FORMULATION OF DUAL CYCLOXYGENASE (COX) AND LIPOXYGENASE (LOX) INHIBITORS FOR MAMMAL SKIN CARE

FIELD OF THE INVENTION

This invention relates generally to a method for the prevention and treatment of diseases and conditions mediated by cyclooxygenase (COX) and lipoxygenase (LOX). Specifically, the present invention relates to a novel composition of matter comprised of a mixture of a blend of two specific classes of compounds --Free-B-Ring flavonoids and flavans-- for use in the prevention and treatment of diseases and conditions of the skin mediated by the COX and LOX pathways. Included in the present invention is a method for the prevention and treatment of COX and LOX mediated diseases and conditions, including but not limited to sun burns, thermal burns, acne, topical wounds, minor inflammatory conditions caused by fungal, microbial and viral infections, vitilago, systemic lupus erythromatosus, psoriasis, carcinoma, melanoma, as well as other mammal skin cancers, skin damage resulting from exposure to ultraviolet (UV) radiation, chemicals, heat, wind and dry environments, wrinkles, saggy skin, lines and dark circles around the eyes, dermatitis and other allergy related conditions of the skin. Use of the composition described herein also affords the benefit of smooth and youthful skin with improved elasticity, reduced and delayed aging, enhanced youthful appearance and texture, and increased flexibility, firmness, smoothness and suppleness.

BACKGROUND OF THE INVENTION

Sunlight has a significant effect on the skin causing premature aging, skin cancer and a host of other skin changes such as erythema and tanning. The majority of the damage caused by sunlight is attributed to ultraviolet (UV) radiation, which has a wavelength from 200 nm to 400 nm. Ultraviolet radiation is divided into three categories, UVA, UVB or UVC, depending on wavelength. UVA, which has a wavelength range from 320-400 nm, can cause tanning and mild sunburn. UVB, which has a wavelength range from 290-320 nm, can cause sunburn and stimulate pigmentation. UVC, which has a wavelength range from 100-290 nm, can cause damage but not tanning. Exposure of the skin to UV radiation induces biphasic reactions. Thus, upon initial exposure an immediate erythema reaction occurs, which is a weak reaction that fades within 30 minutes. A

delayed erythema reaction occurs after 2-5 hours of exposure and peaks around 10-24 hours. Enhanced prostaglandin and leukotriene production are the major mechanisms of action for UV, sun and chemical/thermal caused erythema. (Wang (2002) Adv. Dermatol. 18:247).

The liberation and metabolism of arachidonic acid (AA) from the cell membrane results in the generation of pro-inflammatory metabolites by several different pathways. Arguably, two of the most important pathways to inflammation are mediated by the enzymes lipoxygenase (LOX) and cyclooxygenase (COX). These are parallel pathways that result in the generation of leukotrienes and prostaglandins, respectively, which play important roles in the initiation and progression of the inflammatory response. These vasoactive compounds are chemotaxins, which both promote infiltration of inflammatory cells into tissues and serve to prolong the inflammatory response. Consequently, the enzymes responsible for generating these mediators of inflammation have become the targets in the current invention to develop topically administered therapeutic agents aimed at the dual inhibition of inflammation resulting from both pathways which contribute to the physiological and pathological processes of diseases and conditions such as sun burn, thermal burns, scald, acne, topical wounds, lupus erythromatosus, psoriasis, carcinoma, melanoma, and other mammalian skin cancers.

Inhibition of the COX enzyme is the mechanism of action attributed to most non-steroidal anti-inflammatory drugs (NSAIDS). There are two distinct isoforms of the COX enzyme (COX-1 and COX-2), which share approximately 60% sequence homology, but differ in expression profiles and function. COX-1 is a constitutive form of the enzyme that has been linked to the production of physiologically important prostaglandins, which help regulate normal physiological functions, such as platelet aggregation, protection of cell function in the stomach and maintenance of normal kidney function. (Dannhardt and Kiefer (2001) Eur. J. Med. Chem. 36:109-26). The second isoform, COX-2, is a form of the enzyme that is inducible by pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and other growth factors. (Herschmann (1994) Cancer Metastasis Rev. 134:241-56; Xie *et al.* (1992) Drugs Dev. Res. 25:249-65). This isoform catalyzes the production of prostaglandin E_2 (PGE2) from arachidonic acid (AA). Inhibition of COX is responsible for the anti-inflammatory activity of conventional NSAIDs.

Inhibitors that demonstrate dual specificity for COX and LOX would have the obvious benefit of inhibiting multiple pathways of arachidonic acid metabolism. Such inhibitors would block the inflammatory effects of prostaglandins (PG), as well as, those of multiple leukotrienes (LT) by limiting their production. This includes the vasodilation, vasopermeability and chemotactic effects of PGE2, LTB4, LTD4 and LTE4, also known as the slow reacting substance of anaphalaxis. Of these, LTB4 has the most potent chemotactic and chemokinetic effects. (Moore (1985) in Prostanoids: pharmacological, physiological and clinical relevance, Cambridge University Press, N.Y., pp. 229-230).

Because the mechanism of action of COX inhibitors overlaps that of most conventional NSAID's, COX inhibitors are used to treat many of the same symptoms, including pain and swelling associated with inflammation in transient conditions and chronic diseases in which inflammation plays a critical role. Transient conditions include treatment of inflammation associated with minor abrasions or contact dermatitis, as well as, skin conditions that are directly associated with the prostaglandin and leukotriene pathways, such as skin hyperpigmentation, age spots, vitilago, systemic lupus erythromatosus, psoriasis, carcinoma, melanoma, and other mammalian skin cancers. The use of COX inhibitors has been expanded to include diseases, such as systemic lupus erythromatosus (SLE) (Goebel *et al.* (1999) Chem. Res. Toxicol. 12:488-500; Patrono *et al.* (1985) J. Clin. Invest. 76:1011-1018), as well as, rheumatic skin conditions, such as scleroderma. COX inhibitors are also used for the relief of inflammatory skin conditions that are not of rheumatic origin, such as psoriasis, in which reducing the inflammation resulting from the overproduction of prostaglandins could provide a direct benefit. (Fogh *et al.* (1993) Acta Derm Venerologica 73:191-193). Recently over expression of 5-lipoxygenase in the skin of patients with system sclerosis has been reported. This has led to the suggestion that the LOX pathway may be of significance in the pathogenesis of system sclerosis and may represent a valid therapeutic target. (Kowal-Bielecka (2001) Arthritis Rheum. 44(8):1865). Finally, the increased enzymatic activity of both the COX-2 and 5-LOX at the site of allergen injections suggests the potential for using dual COX/LOX inhibitors to treat the symptoms of both the early and late phases of the skin allergic response. (Church (2002) Clin. Exp. Allergy. 32(7):1013).

Topical application of a selective cyclooxygenase inhibitor has been shown to suppress UVB mediated cutaneous inflammation following both acute and long-term

exposure. Additionally, edema, dermal neutrophil infiltration and activation, PGE2 levels and the formation of sunburn cells were reduced by the topical application of a COX inhibitor. (Wilgus (2000) Prostaglandins Other Lipid Mediat. 62(4):367). The COX inhibitor Celebrex™ has been shown to reduce the effects of UV induced inflammation when administered systematically (Wilgus *et al.* (2002) Adv. Exp. Med. Biol. 507:85), and topically (Wilgus *et al.* (2000) Protaglandins Other Lipid Mediat. 62:367). In animal models, the known COX inhibitor aspirin and various lipoxygenase inhibitors exhibited vasoprotective activity against inflammation and vasodepression resulting from UV irradiation. (Kuhn (1988) Biomed. Biochim. Acta. 47:S320). Acute or long-term chronic UV exposure causes skin damage and photoageing that are characterized by degradation of collagen and accumulation of abnormal elastin in the superficial dermis. A dual COX/LOX inhibitor can be utilized to prevent and treat collagen degradation caused by inflammatory infiltration by significantly reducing the vasodilating, vasopermeability, chemotactic and chemotaxins - prostaglandins (PG), as well as, those of multiple leukotrienes (LT). (Bosset (2003) Br. J. Dermatol. 149(4):826; Hase (2000) Br. J. Dermatol. 142(2):267). Additionally, chemically induced oxidative stress in mouth skin can be inhibited by separately administrating COX and LOX inhibitors to reduce leukocyte adhesion, infiltration and H_2O_2 generation. (Nakamura (2003) Free Radical Biol. Med. 35(9):997).

In addition to their use as anti-inflammatory agents, another potential role for COX inhibitors is in the treatment of cancer. Over expression of COX has been demonstrated in various human malignancies and inhibitors of COX have been shown to be efficacious in the treatment of animals with skin tumors. While the mechanism of action is not completely understood, the over expression of COX has been shown to inhibit apoptosis and increase the invasiveness of tumorgenic cell types. (Dempke *et al.* (2001) J. Can. Res. Clin. Oncol. 127:411-17; Moore and Simmons (2000) Current Med. Chem. 7:1131-1144). Up regulated COX production has been implicated in the generation of actinic keratosis and squamous cell carcinoma in skin. Increased amounts of COX were also found in lesions produced by DNA damage. (Buckman *et al.* (1998) Carcinogenesis 19:723). Therefore, control of expression or protein function of COX would seem to lead to a decrease in the inflammatory response and the eventual progression to cancer. In fact, COX inhibitors such as indomethacin and Celebrex™ have been found to be effective in

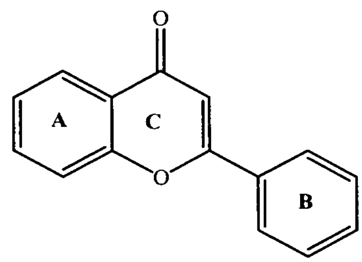
treating UV induced erythema and tumor formation. (Fischer (1999) Mol. Carcinog. 25:231; Pentland (1999) Carcinogenesis 20:1939). Recently, the over expression of lipoxygenase has also been shown to be related to epidermal tumor development (Muller (2002) Cancer Res. 62(16):4610) and melanoma carcinogenesis (Winer (2002) Melanoma Res. 12(5):429). The arachidonic acid (AA) metabolites generated from lipoxygenase pathways play important roles in tumor growth related signal transduction suggesting that that the inhibition of lipoxygenase pathways should be a valid target to prevent cancer progression. (Cuendet (2000) Drug Metabol Drug Interact 17(4):109; Steele (2003) Mutat Res. 523-524:137). Thus, the use of therapeutic agents having dual COX/LOX inhibitory activity offers significant advantages in the chemoprevention of cancer.

Prostaglandins and leukotrienes also play important roles in the physiological and pathological processes of wounds, burns, scald, acne, microbial infections, dermatitis, and many other diseases and conditions of the skin. The activation of a pro-inflammatory cascade after thermal or chemical burns with significantly elevated cyclooxygenase and lipoxygenase activities are well documented and play an important role in the development of subsequent severe symptoms and immune dysfunction that may lead to multiple organ failure. (Schwacha (2003) Burns 29(1):1; He (2001) J. Burn Care Rehabil. 22(1):58).

Acne is a disease of the pilosebaceous unit with abnormalities in sebum production, follicular epithelial desquamation, bacterial proliferation and inflammation. The inflammatory properties of acne can be detected by polarized light photography and utilized for clinical diagnosis, including an evaluation of the extent of the acne and also to determine the effectiveness of therapy. (Phillips (1997) J. Am. Acad. Dermatol. 37(6):948). Current therapeutic agents for the prevention and treatment of acne include anti-inflammatory agents, like retinoids, antimicrobial agents and hormonal drugs. (Leyden (2003) J. Am. Acad. Dermatol. 49(3 Suppl):S200). Topical application of anti-inflammatory drugs, such as retinoids (Millikan (2003) J. Am. Acad. Dermatol. 4(2):75) and the COX inhibitor salicylic acid (Lee (2003) Dermatol Surg 29(12):1196) have been clinically demonstrated as an effective and safe therapy for the treatment of acne. Additionally, the use of nonsteroidal anti-inflammatory drugs (NSAIDs) are well documented as therapeutic agents for common and uncommon dermatoses, including acne, psoriasis, sun burn, erythema nodosum, cryoglobulinemia, Sweet's syndrome, systemic

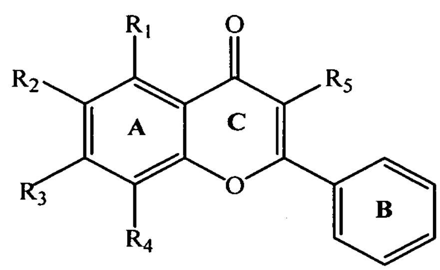
mastocytosis, urticarial, liverdoid and nodular vasculitis. (Friedman (2002) J. Cutan Med. Surg. 6(5):449).

Flavonoids or bioflavonoids are a widely distributed group of natural products, which have been reported to have antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombic and vasodilatory activity. The structural unit common to this group of compounds includes two benzene rings on either side of a 3-carbon ring as illustrated by the following general structural formula:



Various combinations of hydroxyl groups, sugars, oxygen and methyl groups attached to this general three ring structure create the various classes of flavonoids, which include flavanols, flavones, flavan-3-ols (catechins), anthocyanins and isoflavones.

Free-B-Ring flavones and flavonols are a specific class of flavonoids, which have no substituent groups on the aromatic B ring (referred to herein as Free-B-Ring flavonoids), as illustrated by the following general structure:



wherein

R_1, R_2, R_3, R_4, and R_5 are independently selected from the group consisting of -H, -OH, -SH, OR, -SR, $-NH_2$, -NHR, $-NR_2$, $-NR_3^+X^-$, a carbon, oxygen, nitrogen or sulfur, glycoside of a single or a combination of multiple sugars including, but not limited to aldopentoses, methyl-aldopentose, aldohexoses, ketohexose and their chemical derivatives thereof;

wherein

R is an alkyl group having between 1-10 carbon atoms; and

X is selected from the group of pharmaceutically acceptable counter anions including, but not limited to hydroxyl, chloride, iodide, fluoride, sulfate, phosphate, acetate, carbonate, etc.

Free-B-Ring flavonoids are relatively rare. Out of 9,396 flavonoids synthesized or isolated from natural sources, only 231 Free-B-Ring flavonoids are known (The Combined Chemical Dictionary, Chapman & Hall/CRC, Version 5:1 June 2001). Free-B-Ring flavonoids have been reported to have diverse biological activity. For example, galangin (3,5,7-trihydroxyflavone) acts as antioxidant and free radical scavenger and is believed to be a promising candidate for anti-genotoxicity and cancer chemoprevention. (Heo *et al.* (2001) Mutat. Res. 488(2):135-150). It is an inhibitor of tyrosinase monophenolase (Kubo *et al.* (2000) Bioorg. Med. Chem. 8(7):1749-1755), an inhibitor of rabbit heart carbonyl reductase (Imamura *et al.* (2000) J. Biochem. 127(4):653-658), has antimicrobial activity (Afolayan and Meyer (1997) Ethnopharmacol. 57(3):177-181) and antiviral activity (Meyer *et al.* (1997) J. Ethnopharmacol. 56(2):165-169). Baicalein and two other Free-B-Ring flavonoids, have antiproliferative activity against human breast cancer cells. (So *et al.* (1997) Cancer Lett. 112(2):127-133).

Typically, flavonoids have been tested for biological activity randomly based upon their availability. Occasionally, the requirement of substitution on the B-ring has been emphasized for specific biological activity, such as the B-ring substitution required for high affinity binding to p-glycoprotein (Boumendjel *et al.* (2001) Bioorg. Med. Chem. Lett. 11(1):75-77); cardiotonic effect (Itoigawa *et al.* (1999) J. Ethnopharmacol. 65(3): 267-272), protective effect on endothelial cells against linoleic acid hydroperoxide-induced toxicity (Kaneko and Baba (1999) Biosci Biotechnol. Biochem 63(2):323-328), COX-1 inhibitory activity (Wang (2000) Phytomedicine 7:15-19) and prostaglandin endoperoxide synthase (Kalkbrenner *et al.* (1992) Pharmacology 44(1):1-12). Only a few publications have mentioned the significance of the unsubstituted B ring of the Free-B-Ring flavonoids. One example, is the use of 2-phenyl flavones, which inhibit NADPH quinone acceptor oxidoreductase, as potential anticoagulants. (Chen *et al.* (2001) Biochem. Pharmacol. 61(11):1417-1427).

The mechanism of action with respect to the anti-inflammatory activity of various Free-B-Ring flavonoids has been controversial. The anti-inflammatory activity of the Free-B-Ring flavonoids, chrysin (Liang *et al.* (2001) FEBS Lett. 496(1):12-18), wogonin

(Chi *et al.* (2001) Biochem. Pharmacol. 61:1195-1203) and halangin (Raso *et al.* (2001) Life Sci. 68(8):921-931), has been associated with the suppression of inducible cyclooxygenase and nitric oxide synthase via activation of peroxisome proliferator activated receptor gamma (PPARγ) and influence on degranulation and AA release. (Tordera *et al.* (1994) Z. Naturforsch [C] 49:235-240). It has been reported that oroxylin, baicalein and wogonin inhibit 12-lipoxygenase activity without affecting cyclooxygenase. (You *et al.* (1999) Arch. Pharm. Res. 22(1):18-24). More recently, the anti-inflammatory activity of wogonin, baicalin and baicalein has been reported as occurring through inhibition of inducible nitric oxide synthase and *cox*-2 gene expression induced by nitric oxide inhibitors and lipopolysaccharide. (Chen *et al.* (2001) Biochem. Pharmacol. 61(11):1417-1427). It has also been reported that oroxylin acts via suppression of NFκB activation. (Chen *et al.* (2001) Biochem. Pharmacol. 61(11):1417-1427). Finally, wogonin reportedly inhibits inducible PGE2 production in macrophages. (Wakabayashi and Yasui (2000) Eur. J. Pharmacol. 406(3):477-481).

Inhibition of the phosphorylation of mitrogen-activated protein kinase and inhibition of Ca^{2+} ionophore A23187 induced PGE_2 release by baicalein has been reported as the mechanism of anti-inflammatory activity of *Scutellariae radix.* (Nakahata *et al.* (1999) Nippon Yakurigaku Zasshi, 114, Supp. 11:215P-219P; Nakahata *et al.* (1998) Am. J. Chin Med. 26:311-323). Baicalin from *Scutellaria baicalensis*, reportedly inhibits superantigenic staphylococcal exotoxins stimulated T-cell proliferation and production of IL-1β, IL-6, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). (Krakauer *et al.* (2001) FEBS Lett. 500:52-55). Thus, the anti-inflammatory activity of baicalin has been associated with inhibiting the pro-inflammatory cytokines mediated signaling pathways activated by superantigens. However, it has also been proposed that the anti-inflammatory activity of baicalin is due to the binding of a variety of chemokines, which limits their biological activity. (Li *et al.* (2000) Immunopharmacology 49:295-306). Recently, the effects of baicalin on adhesion molecule expression induced by thrombin and thrombin receptor agonist peptide (Kimura *et al.* (2001) Planta Med. 67:331-334), as well as, the inhibition of mitogen-activated protein kinase cascade (MAPK) (Nakahata *et al.* (1999) Nippon Yakurigaku Zasshi, 114, Supp 11:215P-219P; Nakahata *et al.* (1998) Am. J. Chin Med. 26:311-323) have been reported.

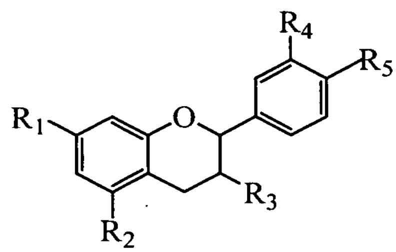
The Chinese medicinal plant, *Scutellaria baicalensis* contains significant amounts of Free-B-Ring flavonoids, including baicalein, baicalin, wogonin and baicalenoside. Traditionally, this plant has been used to treat a number of conditions including clearing away heat, purging fire, dampness-warm and summer fever syndromes; polydipsia resulting from high fever; carbuncle, sores and other pyogenic skin infections; upper respiratory infections, such as acute tonsillitis, laryngopharyngitis and scarlet fever; viral hepatitis; nephritis; pelvitis; dysentery; hematemesis and epistaxis. This plant has also traditionally been used to prevent miscarriage. (Encyclopedia of Chinese Traditional Medicine, ShangHai Science and Technology Press, ShangHai, China, 1998). Clinically *Scutellaria* is now used to treat conditions such as pediatric pneumonia, pediatric bacterial diarrhea, viral hepatitis, acute gallbladder inflammation, hypertension, topical acute inflammation, resulting from cuts and surgery, bronchial asthma and upper respiratory infections. (Encyclopedia of Chinese Traditional Medicine, ShangHai Science and Technology Press, ShangHai, China, 1998). The pharmacological efficacy of *Scutellaria* roots for treating bronchial asthma is reportedly related to the presence of Free-B-Ring flavonoids and their suppression of eotaxin associated recruitment of eosinophils. (Nakajima *et al.* (2001) Planta Med. 67(2):132-135).

To date, a number of naturally occurring Free-B-Ring flavonoids have been commercialized for varying uses. For example, liposome formulations of *Scutellaria* extracts have been utilized for skin care (U.S. Pat. Nos. 5,643,598; 5,443,983). Baicalin has been used for preventing cancer, due to its inhibitory effects on oncogenes (U.S. Pat. No. 6,290,995). Baicalin and other compounds have been used as antiviral, antibacterial and immunomodulating agents (U.S. Pat. No. 6,083,921 and WO98/42363) and as natural anti-oxidants (WO98/49256 and Poland Pub. No. 9,849,256). *Scutellaria baicalensis* root extract has been formulated as a supplemental sun screen agent with additive effects of the cumulative SPFs of each individual component in a topical formulation (WO98/19651). Chrysin has been used for its anxiety reducing properties (U.S. Pat. No. 5,756,538). Anti-inflammatory flavonoids are used for the control and treatment of anorectal and colonic diseases (U.S. Pat. No. 5,858,371), and inhibition of lipoxygenase (U.S. Pat. No. 6,217,875). These compounds are also formulated with glucosamine collagen and other ingredients for repair and maintenance of connective tissue (U.S. Pat. No. 6,333,304). Flavonoid esters constitute active ingredients for cosmetic compositions (U.S. Patent No.

6,235,294). U.S. Application Serial No. 10/091,362, filed March 1, 2002, entitled "Identification of Free-B-Ring Flavonoids as Potent COX-2 Inhibitors," and U.S. Application Serial No. 10/427,746, filed July 22, 2003, entitled "Formulation of a Mixture of Free-B-Ring Flavonoids and Flavans as a Therapeutic Agent" both disclose a method for inhibiting the cyclooxygenase enzyme COX-2 by administering a composition comprising a Free-B-Ring flavonoid or a composition containing a mixture of Free-B-Ring flavonoids to a host in need thereof. This is the first report of a link between Free-B-Ring flavonoids and COX-2 inhibitory activity. These applications are specifically incorporated herein by reference in their entirety.

Japanese Pat. No. 63027435, describes the extraction, and enrichment of baicalein and Japanese Pat. No. 61050921 describes the purification of baicalin.

Flavans include compounds illustrated by the following general structure:



wherein

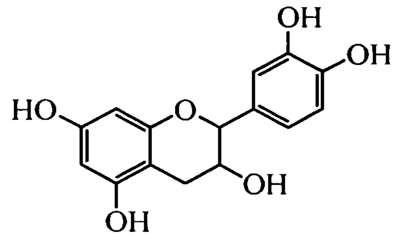
R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of -H, -OH, -SH, $-OCH_3$, $-SCH_3$, -OR, -SR, $-NH_2$, -NRH, $-NR_2$, $-NR_3^+X^-$, esters of the mentioned substitution groups, including, but not limited to, gallate, acetate, cinnamoyl and hydroxyl-cinnamoyl esters, trihydroxybenzoyl esters and caffeoyl esters, and their chemical derivatives thereof; a carbon, oxygen, nitrogen or sulfur glycoside of a single or a combination of multiple sugars including, but not limited to, aldopentoses, methyl aldopentose, aldohexoses, ketohexose and their chemical derivatives thereof; dimer, trimer and other polymerized flavans;

wherein

R is an alkyl group having between 1-10 carbon atoms; and

X is selected from the group of pharmaceutically acceptable counter anions including, but not limited to hydroxyl, chloride, iodide, sulfate, phosphate, acetate, fluoride, and carbonate, etc.

Catechin is a flavan, found primarily in green tea, having the following structure:



Catechin

Catechin works both alone and in conjunction with other flavonoids found in tea, and has both antiviral and antioxidant activity. Catechin has been shown to be effective in the treatment of viral hepatitis. It also appears to prevent oxidative damage to the heart, kidney, lungs and spleen and has been shown to inhibit the growth of stomach cancer cells.

Catechin and its isomer epicatechin inhibit prostaglandin endoperoxide synthase with an IC_{50} value of 40 μM. (Kalkbrenner *et al.* (1992) Pharmacol. 44:1-12). Five flavan-3-ol derivatives, including (+)-catechin and gallocatechin, isolated from four plant species: *Atuna racemosa*, *Syzygium carynocarpum*, *Syzygium malaccense* and *Vantanea peruviana*, exhibit equal to weaker inhibitory activity against COX-2, relative to COX-1, with IC_{50} values ranging from 3.3 μM to 138 μM (Noreen *et al.* (1998) Planta Med. 64:520-524). (+)-Catechin, isolated from the bark of *Ceiba pentandra*, inhibits COX-1 with an IC_{50} value of 80 μM (Noreen *et al.* (1998) J. Nat. Prod. 61:8-12). Commercially available pure (+)-catechin inhibits COX-1 with an IC_{50} value of around 183 to 279 μM depending upon the experimental conditions, with no selectivity for COX-2. (Noreen *et al.* (1998) J. Nat. Prod. 61:1-7).

Green tea catechin, when supplemented into the diets of Sprague dawley male rats, lowered the activity level of platelet PLA_2 and significantly reduced platelet cyclooxygenase levels. (Yang *et al.* (1999) J. Nutr. Sci. Vitaminol. 45:337-346). Catechin and epicatechin reportedly weakly suppress *cox*-2 gene transcription in human colon cancer DLD-1 cells (IC_{50} = 415.3 μM). (Mutoh *et al.* (2000) Jpn. J. Cancer Res. 91:686-691). The neuroprotective ability of (+)-catechin from red wine results from the antioxidant properties of catechin, rather than inhibitory effects on intracellular enzymes, such as cyclooxygenase, lipoxygenase, or nitric oxide synthase (Bastianetto *et al.* (2000) Br. J. Pharmacol. 131:711-720). Catechin derivatives purified from green and black tea, such as epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and theaflavins showed inhibition of cyclooxygenase and lipoxygenase dependent

metabolism of AA in human colon mucosa and colon tumor tissues (Hong *et al.* (2001) Biochem. Pharmacol. 62:1175-1183) and induce *cox*-2 expression and PGE_2 production (Park *et al.* (2001) Biochem. Biophys. Res. Commun. 286:721-725). Epiafzelechin isolated from the aerial parts of *Celastrus orbiculatus* exhibited dose-dependent inhibition of COX-1 activity with an IC_{50} value of 15 μM and also demonstrated anti-inflammatory activity against carrageenin-induced mouse paw edema following oral administration at a dosage of 100 mg/kg. (Min *et al.* (1999) Planta Med. 65:460-462).

Acacia is a genus of leguminous trees and shrubs. The genus *Acacia* includes more than 1000 species belonging to the family of Leguminosae and the subfamily of Mimosoideae. *Acacias* are distributed worldwide in tropical and subtropical areas of Central and South America, Africa, parts of Asia, as well as, Australia, which has the largest number of endemic species. *Acacias* are very important economically, providing a source of tannins, gums, timber, fuel and fodder. Tannins, which are isolated primarily from bark, are used extensively for tanning hides and skins. Some *Acacia* barks are also used for flavoring local spirits. Some indigenous species like *A. sinuata* also yield saponins, which are any of various plant glucosides that form soapy lathers when mixed and agitated with water. Saponins are used in detergents, foaming agents and emulsifiers. The flowers of some *Acacia* species are fragrant and used to make perfume. The heartwood of many A*cacias* is used for making agricultural implements and also provides a source of firewood. *Acacia* gums find extensive use in medicine and confectionary and as sizing and finishing materials in the textile industry.

To date, approximately 330 compounds have been isolated from various *Acacia* species. Flavonoids are the major class of compounds isolated from *Acacias*. Approximately 180 different flavonoids have been identified, 111 of which are flavans. Terpenoids are second largest class of compounds isolated from species of the *Acacia* genus, with 48 compounds having been identified. Other classes of compounds isolated from *Acacia* include, alkaloids (28), amino acids/peptides (20), tannins (16), carbohydrates (15), oxygen heterocycles (15) and aliphatic compounds (10). (Buckingham, The Combined Chemical Dictionary, Chapman & Hall CRC, version 5:2, Dec. 2001).

Phenolic compounds, particularly flavans are found in moderate to high concentrations in all *Acacia* species. (Abdulrazak *et al.* (2000) Journal of Animal Sciences. 13:935-940). Historically, most of the plants and extracts of the *Acacia* genus

have been utilized as astringents to treat gastrointestinal disorders, diarrhea, indigestion and to stop bleeding. (Vautrin (1996) Universite Bourgogne (France) European abstract 58-01C:177; Saleem *et al.* (1998) Hamdard Midicus. 41:63-67). The bark and pods of *Acacia arabica Willd.* contain large quantities of tannins and have been utilized as astringents and expectorants. (Nadkarni (1996) India Materia Medica, Bombay Popular Prakashan, pp. 9-17). Diarylpropanol derivatives, isolated from stem bark of *Acacia tortilis* from Somalia, have been reported to have smooth muscle relaxing effects. (Hagos *et al.* (1987) Planta Medica. 53:27-31, 1987). It has also been reported that terpenoid saponins isolated from *Acacia victoriae* have an inhibitory effect on dimethylbenz(a)anthracene-induced murine skin carcinogenesis (Hanausek *et al.* (2000) Proceedings American Association for Cancer Research Annual Meeting 41:663) and induce apotosis (Haridas *et al.* (2000) Proceedings American Association for Cancer Research Annual Meeting. 41:600). Plant extracts from *Acacia nilotica* have been reported to have spasmogenic, vasoconstrictor and anti-hypertensive activity (Amos *et al.* (1999) Phytotherapy Research 13:683-685; Gilani *et al.* (1999) Phytotherapy Research. 13:665-669), and antiplatelet aggregatory activity (Shah *et al.* (1997) General Pharmacology. 29:251-255). Anti-inflammatory activity has been reported for *A. nilotica*. It was speculated that flavonoids, polysaccharides and organic acids were potential active components. (Dafallah and Al-Mustafa (1996) American Journal of Chinese Medicine. 24:263-269). To date, the only reported 5-lipoxygenase inhibitor isolated from *Acacia* is a monoterpenoidal carboxamide (Seikine *et al.* (1997) Chemical and Pharmaceutical Bulletin. 45:148-11).

The extract from the bark of *Acacia* has been patented in Japan for external use as a whitening agent (Abe, JP10025238), as a glucosyl transferase inhibitor for dental applications (Abe, JP07242555), as a protein synthesis inhibitor (Fukai, JP 07165598), as an active oxygen scavenging agent for external skin preparations (Honda, JP 07017847, Bindra U.S. Pat. No. 6,1266,950), and as a hyaluronidase inhibitor for oral consumption to prevent inflammation, pollinosis and cough (Ogura, JP 07010768).

To date, Applicant is unaware of any reports of a formulation combining only Free-B-Ring-Flavonoids and flavans as the primary biologically active components for the dual inhibition of the COX/LOX enzymes that yield significant benefit to mammal skin conditions.

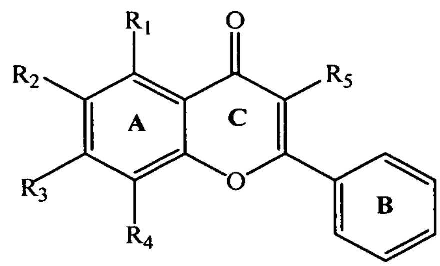
SUMMARY OF THE INVENTION

The present invention includes methods that are effective in simultaneously inhibiting both the cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, for use in the prevention and treatment of diseases and conditions related to the skin. The method for the simultaneous dual inhibition of the COX and LOX enzymes is comprised administering, preferably topically, a composition comprised of a mixture of Free-B-Ring flavonoids and flavans synthesized and/or isolated from a single plant or multiple plants to a host in need thereof. This composition of matter is referred to herein as Soliprin™. The efficacy of this method was demonstrated with purified enzymes, in different cell lines, in multiple animal models and eventually in a human clinical study. The ratio of the Free-B-Ring flavonoids to flavans in the composition can be in the range of 99.9:0.1 of Free-B-Ring flavonoids:flavans to 0.1:99.9 Free-B-Ring flavonoids:flavans. In specific embodiments of the present invention, the ratio of Free-B-Ring flavonoids to flavans is selected from the group consisting of approximately 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. In a preferred embodiment of this invention, the ratio of Free-B-Ring flavonoids:flavans in the composition of matter is 80:20. In a preferred embodiment, the Free-B-Ring flavonoids are isolated from a plant or plants in the *Scutellaria* genus of plants and the flavans are isolated from a plant or plants in the *Acacia* genus of plants.

The present invention also includes methods for the prevention and treatment of COX and LOX mediated diseases and conditions of the skin. The method for preventing and treating COX and LOX mediated diseases and conditions of the skin is comprised of administering, preferably topically, to a host in need thereof an effective amount of a composition comprising a mixture of Free-B-Ring flavonoids and flavans synthesized and/or isolated from a single plant or multiple plants and a pharmaceutically acceptable carrier. The ratio of the Free-B-Ring flavonoids to flavans in the composition can be in the range of 99.9:0.1 of Free-B-Ring flavonoids:flavans to 0.1:99.9 Free-B-Ring flavonoids:flavans. In specific embodiments of the present invention, the ratio of Free-B-Ring flavonoids to flavans is selected from the group consisting of approximately 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. In a preferred embodiment of this invention, the ratio of Free-B-Ring flavonoids:flavans in the composition of matter is 80:20. In a preferred embodiment, the Free-B-Ring flavonoids are isolated from a plant or

plants in the *Scutellaria* genus of plants and the flavans are isolated from a plant or plants in the *Acacia* genus of plants.

The Free-B-Ring flavonoids, also referred to herein as Free-B-Ring flavones and flavonols, that can be used in accordance with the following invention include compounds illustrated by the following general structure:



wherein

R_1, R_2, R_3, R_4, and R_5 are independently selected from the group consisting of -H, -OH, -SH, OR, -SR, $-NH_2$, -NHR, $-NR_2$, $-NR_3^+X^-$, a carbon, oxygen, nitrogen or sulfur, glycoside of a single or a combination of multiple sugars including, but not limited to aldopentoses, methyl-aldopentose, aldohexoses, ketohexose and their chemical derivatives thereof;

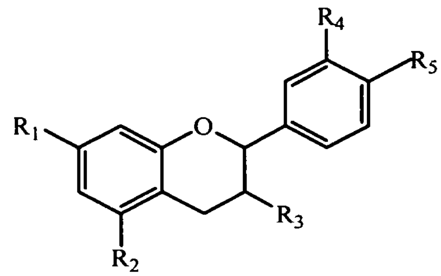
wherein

R is selected from an alkyl group having between 1-10 carbon atoms; and

X is selected from the group of pharmaceutically acceptable counter anions including, but not limited to hydroxyl, chloride, iodide, sulfate, phosphate, acetate, fluoride, carbonate, etc.

The Free-B-Ring flavonoids of this invention may be obtained by synthetic methods or extracted from the family of plants including, but not limited to *Annonaceae, Asteraceae, Bignoniaceae, Combretaceae, Compositae, Euphorbiaceae, Labiatae, Lauranceae, Leguminosae, Moraceae, Pinaceae, Pteridaceae, Sinopteridaceae, Ulmaceae* and *Zingiberacea*. The Free-B-Ring flavonoids can be extracted, concentrated, and purified from the following genus of high plants, including but not limited to *Desmos, Achyrocline, Oroxylum, Buchenavia, Anaphalis, Cotula, Gnaphalium, Helichrysum, Centaurea, Eupatorium, Baccharis, Sapium, Scutellaria, Molsa, Colebrookea, Stachys, Origanum, Ziziphora, Lindera, Actinodaphne, Acacia, Derris, Glycyrrhiza, Millettia, Pongamia, Tephrosia, Artocarpus, Ficus, Pityrogramma, Notholaena, Pinus, Ulmus* and *Alpinia*.

The flavans that can be used in accordance with the following invention include compounds illustrated by the following general structure:



wherein

R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of H, -OH, -SH, $-OCH_3$, $-SCH_3$, -OR, -SR, $-NH_2$, -NRH, $-NR_2$, $-NR_3^+X^-$, esters of the mentioned substitution groups, including, but not limited to, gallate, acetate, cinnamoyl and hydroxyl-cinnamoyl esters, trihydroxybenzoyl esters and caffeoyl esters; thereof carbon, oxygen, nitrogen or sulfur glycoside of a single or a combination of multiple sugars including, but not limited to, aldopentoses, methyl aldopentose, aldohexoses, ketohexose and their chemical derivatives thereof; dimer, trimer and other polymerized flavans;

wherein

R is selected from an alkyl group having between 1-10 carbon atoms; and

X is selected from the group of pharmaceutically acceptable counter anions including, but not limited to hydroxyl, chloride, iodide, sulfate, phosphate, acetate, fluoride, carbonate, etc.

The flavans of this invention may be obtained from a plant or plants selected from the genus of *Acacia*. In a preferred embodiment, the plant is selected from the group consisting of *Acacia catechu, Acacia concinna, Acacia farnesiana, Acacia Senegal, Acacia speciosa, Acacia arabica, A. caesia, A. pennata, A. sinuata. A. mearnsii, A. picnantha, A. dealbata, A. auriculiformis, A. holoserecia* and *A. mangium.*

In one embodiment, the present invention includes a method for preventing and treating a number of COX and LOX mediated diseases and conditions of the skin including, but not limited to sun burns, thermal burns, acne, topical wounds, minor inflammatory conditions caused by fungal, microbial and viral infections, vitilago, systemic lupus erythromatosus, psoriasis, carcinoma, melanoma, as well as other mammal skin cancers. In another embodiment the present invention includes a method for preventing and treating skin damage resulting from exposure to ultraviolet (UV) radiation,

chemicals, heat, wind and dry environments. In yet another embodiment the present invention includes a method for preventing and treating wrinkles, saggy skin, lines and dark circles around the eyes, dermatitis and other allergy related conditions of the skin.

The present invention further includes therapeutic compositions comprising the therapeutic agents of the present invention. In addition to their use for the prevention and treatment of the above described diseases and conditions of the skin, the therapeutic compositions described herein can also be used to sooth sensitive skin and to provide smooth and youthful skin with improved elasticity, reduced and delayed aging, enhanced youthful appearance and texture, and increased flexibility, firmness, smoothness and suppleness.

The method of prevention and treatment according to this invention comprises administering topically to a host in need thereof a therapeutically effective amount of the formulated Free-B-Ring flavonoids and flavans isolated from a single source or multiple sources. The purity of the individual and/or a mixture of multiple Free-B-Ring flavonoids and flavans includes, but is not limited to 0.01% to 100%, depending on the methodology used to obtain the compound(s). In a preferred embodiment, doses of the mixture of Free-B-Ring flavonoids and flavans containing the same are an efficacious, nontoxic quantity generally selected from the range of 0.001% to 100% based on total weight of the topical formulation. Persons skilled in the art using routine clinical testing are able to determine optimum doses for the particular ailment being treated.

The present invention includes an evaluation of different compositions of Free-B-Ring flavonoids and flavans using enzymatic and *in vivo* models to optimize the formulation and obtain the desired physiological activity. The efficacy and safety of this formulation is also demonstrated in human clinical studies. The compositions of this invention can be administered by any method known to one of ordinary skill in the art. The modes of administration include, but are not limited to, enteral (oral) administration, parenteral (intravenous, subcutaneous, and intramuscular) administration and topical application. In the preferred embodiment the method of treatment according to this invention comprises administering topically to a host in need thereof a therapeutically effective amount of a mixture of Free-B-Ring flavonoids and flavans synthesized and/or isolated from a single plant or multiple plants.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts graphically a profile of the inhibition of COX-1 and COX-2 by a standardized Free-B-Ring flavonoid extract (83% baicalin based on HPLC) which was isolated from *Scutellaria baicalensis*. The extract was examined for its inhibition of the peroxidase activity of recombinant ovine COX-1 (♦) and ovine COX-2 (■). The data is presented as percent inhibition vs. inhibitor concentration (μg/mL). The IC_{50} for COX-1 was calculated as 0.24 μg/mL/unit of enzyme while the IC_{50} for COX-2 was calculated as 0.48 μg/mL/unit.

Figure 2 depicts graphically a profile of the inhibition of COX-1 and COX-2 by the purified component baicalin which was isolated from *Scutellaria baicalensis*. The compound was examined for its inhibition of the peroxidase activity of recombinant ovine COX-1 (♦) and ovine COX-2 (■). The data is presented as percent inhibition vs. inhibitor concentration (μg/mL). The IC_{50} for COX-1 was determined to be 0.44 μg/mL/unit of enzyme and the IC_{50} for COX-2 was determined to be 0.28 μg/mL/unit.

Figure 3 depicts graphically a profile of the inhibition of COX-1 and COX-2 by the purified component baicalein isolated from *Scutellaria baicalensis*. The compound was examined for its inhibition of the peroxidase activity of recombinant ovine COX-1 (♦) and ovine COX-2 (■). The data is presented as percent inhibition vs. inhibitor concentration (μg/mL). The IC_{50} for COX-1 was determined to be 0.18 μg/mL/unit of enzyme and the IC_{50} for COX-2 was determined to be 0.28 μg/mL/unit.

Figure 4 depicts graphically a profile of the inhibition of COX-1 and COX-2 by a standardized flavan extract containing 50% total flavans which was isolated from *Acacia catechu*. The extract was examined for its inhibition of the peroxidase activity of recombinant ovine COX-1 (♦) and ovine COX-2 (■). The data is presented as percent inhibition vs. inhibitor concentration (μg/mL). The IC_{50} for COX-1 was calculated as 0.17 μg/mL/unit of enzyme and the IC_{50} for COX-2 was calculated as 0.41 μg/mL/unit.

Figure 5 depicts graphically a profile of the inhibition of COX-1 and COX-2 by the a composition of matter comprised of greater than 90% flavans isolated from *Acacia*

catechu. The composition was examined for its inhibition of the peroxidase activity of recombinant ovine COX-1 (◆) and ovine COX-2 (■). The data is presented as percent inhibition vs. inhibitor concentration (μg/mL). The IC_{50} for COX-1 was calculated as 0.11 μg/mL/unit of enzyme and the IC_{50} for COX-2 was calculated as 0.42 μg/mL/unit.

Figure 6 depicts graphically a profile of the inhibition of COX-1 and COX-2 by a formulation produced by combining an extract of Free-B-Ring flavonoids isolated from the roots of *Scutellaria baicalensis* and an extract of flavans isolated from the bark of *Acacia catechu* in a ratio of 80:20. This composition of matter, referred to hereinafter as Soliprin™, was examined for its inhibition of the peroxidase activity of recombinant ovine COX-1 (◆) and ovine COX-2 (■). The data is presented as percent inhibition vs. inhibitor concentration (μg/mL). The IC_{50} for COX-1 was calculated as 0.76 μg/mL/unit of enzyme and the IC_{50} for COX-2 was calculated as 0.80 μg/mL/unit.

Figure 7 depicts graphically a profile of the inhibition of COX-1 and COX-2 by a formulation produced by combining an extract of Free-B-Ring flavonoids isolated from the roots of *Scutellaria baicalensis* and an extract of flavans isolated from the bark of *Acacia catechu* in a ratio of about 50:50. The composition, Soliprin™, was examined for its inhibition of the peroxidase activity of recombinant ovine COX-1 (◆) and ovine COX-2 (■). The data is presented as percent inhibition vs. inhibitor concentration (μg/mL). The IC_{50} for COX-1 was calculated as 0.38 μg/mL/unit of enzyme and the IC_{50} for COX-2 was determined to be 0.84 μg/mL/unit.

Figure 8 depicts graphically a profile of the inhibition of COX-1 and COX-2 by a formulation produced by combining an extract of Free-B-Ring flavonoids isolated from the roots of *Scutellaria baicalensis* and an extract of flavans isolated from the bark of *Acacia catechu* in a ratio of about 20:80. The composition, Soliprin™, was examined for its inhibition of the peroxidase activity of recombinant ovine COX-1 (◆) and ovine COX-2 (■). The data is presented as percent inhibition vs. inhibitor concentration (μg/mL). The IC_{50} of this composition for COX-1 was 0.18 μg/mL/unit of enzyme and the IC_{50} for COX-2 was 0.41 μg/mL/unit.

Figure 9 depicts graphically a profile of the inhibition of 5-LO by the flavan extract from *Acacia catechu*. The composition was examined for its inhibition of recombinant potato 5-lipoxygenase activity (◆) as described in Example 4. The data is presented as

percent inhibition of assays without inhibitor. The IC_{50} for 5-LO was 1.38 μg/mL/unit of enzyme.

Figure 10 illustrates the High Pressure Liquid Chromatography (HPLC) chromatogram of a typical formulation comprised of a mixture of Free-B-Ring flavonoids isolated from the roots of *Scutellaria baicalensis* and flavans isolated from the bark of *Acacia catechu* in a ratio of 80:20 carried out under the conditions as described in Example 9.

Figure 11 depicts graphically the effect of increasing concentrations of Soliprin™ on the amount of LPS-induced newly synthesized LTB_4 (♦) as determined by ELISA in THP-1 or HT-29 cells (ATCC) as described in Example 10. The Soliprin™ was produced through the combination of standardized extracts of Free-B-Ring flavonoids isolated from the roots of *Scutellaria baicalensis* and flavans isolated from the bark of *Acacia catechu* in a ratio of 80:20. The activity of the Soliprin™ formulation is expressed as % inhibition of induced LTB_4 synthesis.

Figure 12 compares the LTB_4 levels as determined by ELISA that remain in HT-29 cells after treatment with 3 μg/mL Soliprin™ in non-induced cells to treatment with 3 μg/mL ibuprofen as described in Example 10. The Soliprin™ formulation demonstrated 80% inhibition of LTB4 production in the HT-29 cells after two days of treatment.

Figure 13 illustrates graphically ear-swelling data as a measure of inhibition of inflammation as described in Example 11. Soliprin™ produced through the combination of standardized extracts of Free-B-Ring flavonoids isolated from the roots of *Scutellaria baicalensis* and flavans isolated from the bark of *Acacia catechu* in a ratio of 80:20 was compared to untreated mice and mice given indomethacin (1.5 μg/kg) via oral gavage. The data is presented as the difference in micron measurement of the untreated vs. the treated ear lobe for each mouse.

Figure 14 illustrates graphically the effect of 100 mg/kg of Soliprin™, produced through the combination of standardized extracts of Free-B-Ring flavonoids isolated from the roots of *Scutellaria baicalensis* and flavans isolated from the bark of *Acacia catechu* in a ratio of 80:20 on the AA injected ankles of mice (Soliprin™ + arachidonic acid) compared to non-treated mice (no treatment + arachidonic acid), mice without AA

injections (negative control) or mice that were injected with the liquid carrier (vehicle control).

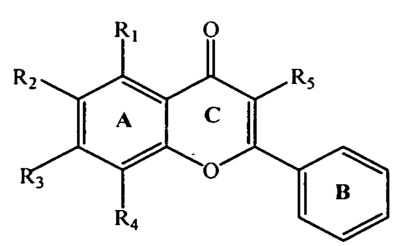
Figure 15 depicts graphically the changes in hairless mice skin erythema scores in different treatment groups as a function of time following irradiation of the mice with UV light as described in Example 12. The mice in Groups B-1, A-1, B-2 and A-2 were treated with Soliprin™ either before (Groups B-1 and B-2) or after (A-1 and A-2) irradiation. The Soliprin™ was produced through the combination of standardized extracts of Free-B-Ring flavonoids isolated from the roots of *Scutellaria baicalensis* and flavans isolated from the bark of *Acacia catechu* in a ratio of 80:20. With reference to Figure 15, it can be seen that topical applications of Soliprin™, both before and after UV radiation, significantly reduced erythema scores as compared with the control group and the group that was administered the standard treatment agent-Sooth-a-caine.

DETAILED DESCRIPTION OF THE INVENTION

Various terms are used herein to refer to aspects of the present invention. To aid in the clarification of the description of the components of this invention, the following definitions are provided.

It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a flavonoid refers to one or more flavonoids. As such, the terms "a" or "an", "one or more" and "at least one" are used interchangeably herein.

"Free-B-Ring Flavonoids" as used herein are a specific class of flavonoids, which have no substitute groups on the aromatic B-ring, as illustrated by the following general structure:



wherein

R_1, R_2, R_3, R_4, and R_5 are independently selected from the group consisting of -H, -OH, -SH, OR, -SR, $-NH_2$, -NHR, $-NR_2$, $-NR_3^+X^-$, a carbon, oxygen, nitrogen or sulfur, glycoside of a single or a combination of multiple sugars including, but not limited to

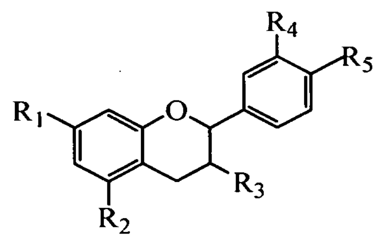
aldopentoses, methyl-aldopentose, aldohexoses, ketohexose and their chemical derivatives thereof;

wherein

R is an alkyl group having between 1-10 carbon atoms; and

X is selected from the group of pharmaceutically acceptable counter anions including, but not limited to hydroxyl, chloride, iodide, sulfate, phosphate, acetate, fluoride, carbonate, etc.

"**Flavans**" as used herein refer to a specific class of flavonoids, which can be generally represented by the following general structure:



wherein

R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of H, -OH,

-SH, $-OCH_3$, $-SCH_3$, -OR, -SR, $-NH_2$, -NRH, $-NR_2$, $-NR_3^+X^-$, esters of substitution groups, including, but not limited to, gallate, acetate, cinnamoyl and hydroxyl-cinnamoyl esters, trihydroxybenzoyl esters and caffeoyl esters and their chemical derivatives thereof; carbon, oxygen, nitrogen or sulfur glycoside of a single or a combination of multiple sugars including, but not limited to, aldopentoses, methyl aldopentose, aldohexoses, ketohexose and their chemical derivatives thereof; dimer, trimer and other polymerized flavans;

wherein

R is an alkyl group having between 1-10 carbon atoms; and

X is selected from the group of pharmaceutically acceptable counter anions including, but not limited to hydroxyl, chloride, iodide, sulfate, phosphate, acetate, fluoride, carbonate, etc.

"**Therapeutic**" as used herein, includes treatment and/or prophylaxis. When used, therapeutic refers to humans as well as other animals.

"Pharmaceutically or therapeutically effective dose or amount" refers to a dosage level sufficient to induce a desired biological result. That result may be the alleviation of the signs, symptoms or causes of a disease or any other alteration of a biological system that is desired.

"Placebo" refers to the substitution of the pharmaceutically or therapeutically effective dose or amount dose sufficient to induce a desired biological that may alleviate the signs, symptoms or causes of a disease with a non-active substance.

A **"host"** or **"patient"** is a living subject, human or animal, into which the compositions described herein are administered. Thus, the invention described herein may be used for veterinary as well as human applications and the terms "patient" or "host" should not be construed in a limiting manner. In the case of veterinary applications, the dosage ranges can be determined as described below, taking into account the body weight of the animal.

Note that throughout this application various citations are provided. Each citation is specifically incorporated herein in its entirety by reference.

The current invention provides methods for the extraction (Example 1, Table 1) of plants that contain Free-B-Ring flavonoids and flavans with organic and aqueous solvents. The crude extracts were assayed for cyclooxygenase inhibitory activity (Example 2, Tables 2 and 3). Purified Free-B-Ring flavonoids and flavans demonstrated inhibitory activity against cyclooxygenase (COX) and lipoxygenase (LOX), respectively, as shown in Examples 3 and 4. Methods for analyzing and quantifying the extracts are described in Examples 5 and 6 and the procedures to generate standardized Free-B-Ring flavonoids and flavans from botanical origins are provided in Examples 7 and 8.

In one embodiment of the present invention, the standardized Free-B-Ring flavonoid extract is comprised of the active compounds having a purity of between 1-99% (by weight) of total Free-B-Ring flavonoids as defined in Examples 1, 2, 5 and 8. Baicalin is the major active component in the extract, which accounts for approximately 50-90% (by weight) of the total Free-B-Ring flavonoids. In a preferred embodiment, the standardized extract contains >70% total Free-B-Ring flavonoids in which >75% of the Free-B-Ring flavonoids is baicalin.

In one embodiment, the standardized flavan extract is comprised of the active compounds having a purity of between 1-99% (by weight) total flavans as defined in

Examples 1, 4, 6 and 7. Catechin is the major active component in the extract and accounts for 50-95% (by weight) of the total flavans. In a preferred embodiment, the standardized flavan extract contains >80% total flavans in which >70% of flavans is catechin.

In one embodiment, Soliprin™ is produced by mixing the above two extracts or synthetic compounds in a ratio from 99:1 to 1:99. The preferred ratios of Free-B-Ring flavonoids to flavans are 80:20 as defined in Example 9 and Table 10 and 15:85 as defined in Example 9.

The concentration of Free-B-Ring flavonoids in Soliprin™ can be from about 1% to 99% and the concentration of flavans in Soliprin™ can be from 99% to 1%. In a preferred embodiment of the invention, the concentration of total Free-B-Ring flavonoids in Soliprin™ is approximately 20% with a baicalin content of approximately 15% of total weight of the Soliprin™; and the concentration of total flavans in Soliprin™ is approximately 75% with a catechin content of approximately 70%. In this embodiment, the total active components (Free-B-Ring flavonoids plus flavans) in Soliprin™ are >90% of the total weight.

The present invention includes methods that are effective in simultaneously inhibiting both the cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, for use in the prevention and treatment of diseases and conditions related to the skin. The method for the simultaneous dual inhibition of the COX and LOX enzymes is comprised of administering, preferably topically a composition comprised of a mixture of Free-B-Ring flavonoids and flavans synthesized and/or isolated from a single plant or multiple plants to a host in need thereof. This composition of matter is referred to herein as Soliprin™. The efficacy of this method was demonstrated with purified enzymes, in different cell lines, in multiple animal models and eventually in a human clinical study. The ratio of the Free-B-Ring flavonoids to flavans in the composition can be in the range of 99.9:0.1 of Free-B-Ring flavonoids:flavans to 0.1:99.9 Free-B-Ring flavonoids:flavans. In specific embodiments of the present invention, the ratio of Free-B-Ring flavonoids to flavans is selected from the group consisting of approximately 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. In a preferred embodiment of this invention, the ratio of Free-B-Ring flavonoids:flavans in the composition of matter is 20:80. In a preferred embodiment, the Free-B-Ring flavonoids are isolated from a plant or plants in the

Scutellaria genus of plants and the flavans are isolated from a plant or plants in the *Acacia* genus of plants.

The present invention also includes methods for the prevention and treatment of COX and LOX mediated diseases and conditions of the skin. The method for preventing and treating COX and LOX mediated diseases and conditions of the skin is comprised of administering, preferably topically, to a host in need thereof an effective amount of a composition comprised of a mixture of Free-B-Ring flavonoids and flavans synthesized and/or isolated from a single plant or multiple plants and a pharmaceutically acceptable carrier. The ratio of the Free-B-Ring flavonoids to flavans in the composition can be in the range of 99.9:0.1 of Free-B-Ring flavonoids:flavans to 0.1:99.9 Free-B-Ring flavonoids:flavans. In specific embodiments of the present invention, the ratio of Free-B-ring flavonoids to flavans is selected from the group consisting of approximately 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. In a preferred embodiment of this invention, the ratio of Free-B-Ring flavonoids:flavans in the composition of matter is 20:80. In a preferred embodiment, the Free-B-Ring flavonoids are isolated from a plant or plants in the *Scutellaria* genus of plants and the flavans are isolated from a plant or plants in the *Acacia* genus of plants.

In one embodiment, the present invention includes a method for preventing and treating a number of COX and LOX mediated diseases and conditions of the skin including, but not limited to sun burns, thermal burns, acne, topical wounds, minor inflammatory conditions caused by fungal, microbial and viral infections, vitilago, systemic lupus erythromatosus, psoriasis, carcinoma, melanoma, as well as other mammal skin cancers. In another embodiment the present invention includes a method for preventing and treating skin damage resulting from exposure to UV radiation, chemicals, heat, wind and dry environments. In yet another embodiment the present invention includes a method for preventing and treating wrinkles, saggy skin, lines and dark circles around the eyes, dermatitis and other allergy related conditions of the skin.

The present invention further includes therapeutic compositions comprising the therapeutic agents of the present invention. In addition to their use for the prevention and treatment of the above described diseases and conditions of the skin, the therapeutic compositions described herein can be used to sooth sensitive skin and to provide smooth

and youthful skin with improved elasticity, reduced and delayed aging, enhanced youthful appearance and texture, and increased flexibility, firmness, smoothness and suppleness.

The Free-B-Ring flavonoids that can be used in accordance with the instant invention include compounds illustrated by the general structure set forth above. The Free-B-Ring flavonoids of this invention may be obtained by synthetic methods or may be isolated from the family of plants including, but not limited to *Annonaceae, Asteraceae, Bignoniaceae, Combretaceae, Compositae, Euphorbiaceae, Labiatae, Lauranceae, Leguminosae, Moraceae, Pinaceae, Pteridaceae, Sinopteridaceae, Ulmaceae,* and *Zingiberacea*. The Free-B-Ring flavonoids can be extracted, concentrated, and purified from the following genus of high plants, including but not limited to *Desmos, Achyrocline, Oroxylum, Buchenavia, Anaphalis, Cotula, Gnaphalium, Helichrysum, Centaurea, Eupatorium, Baccharis, Sapium, Scutellaria, Molsa, Colebrookea, Stachys, Origanum, Ziziphora, Lindera, Actinodaphne, Acacia, Derris, Glycyrrhiza, Millettia, Pongamia, Tephrosia, Artocarpus, Ficus, Pityrogramma, Notholaena, Pinus, Ulmus* and *Alpinia.*

The flavonoids can be found in different parts of plants, including but not limited to stems, stem barks, twigs, tubers, roots, root barks, young shoots, seeds, rhizomes, flowers and other reproductive organs, leaves and other aerial parts. Methods for the isolation and purification of Free-B-Ring flavonoids are described in U.S. Application Serial No. 10/091,362, filed March 1, 2002, entitled "Identification of Free-B-Ring Flavonoids as Potent Cox-2 Inhibitors," which is incorporated herein by reference in its entirety.

The flavans that can be used in accordance with the method of this invention include compounds illustrated by the general structure set forth above. The flavans of this invention are isolated from a plant or plants selected from the *Acacia* genus of plants. In a preferred embodiment, the plant is selected from the group consisting of *Acacia catechu* (*A. catechu*), *A. concinna, A. farnesiana, A. Senegal, A. speciosa, A. arabica, A. caesia, A. pennata, A. sinuata. A. mearnsii, A. picnantha, A. dealbata, A. auriculiformis, A. holoserecia* and *A. mangium*.

The flavans can be found in different parts of plants, including but not limited to stems, stem barks, trunks, trunk barks, twigs, tubers, roots, root barks, young shoots, seeds, rhizomes, flowers and other reproductive organs, leaves and other aerial parts. Methods for the isolation and purification of flavans are described in U.S. Application Serial No.

10/104,477, filed March 22, 2002, entitled "Isolation of a Dual Cox-2 and 5-Lipoxygenase Inhibitor from Acacia," which is incorporated herein by reference in its entirety.

The present invention implements a strategy that combines a series of *in vivo* inflammation and toxicity studies as well as *in vitro* biochemical, cellular, and gene expression screens to identify active plant extracts that specifically inhibit COX and LOX enzymatic activity, impact mRNA gene expression and reduce inflammation. The methods used herein to identify active plant extracts that specifically inhibit COX and LOX are described in Examples 1 and 2, as well as in U.S. Application Serial No. 10/091,362, filed March 1, 2002, entitled "Identification of Free-B-Ring Flavonoids as Potent Cox-2 Inhibitors;" U.S. Application Serial No. 10/104,477, filed March 22, 2002, entitled "Isolation of a Dual Cox-2 and 5-Lipoxygenase Inhibitor from Acacia," and U.S. Application Serial No. 10/427,746, filed April 30, 2003, entitled "Formulation With Dual Cox-2 And 5-Lipoxygenase Inhibitory Activity," each of which is incorporated herein by reference in its entirety.

The biochemical assay, used to measure inhibition of COX, relies on the protein's peroxidase activity in the presence of heme and arachidonic acid. This study which is described in Example 3, showed that the purified Free-B-Ring flavonoids, baicalin and baicalein isolated from *Scutellaria baicalensis* and the flavan extract isolated from *Acacia catechu*, and each individual standardized extract containing high concentrations of Free-B-Ring flavonoids and flavans inhibited COX activity (Figures 1-5). Additionally, compositions having different ratios of each of the individual standardized extracts (i.e., 80:20, 50:50 and 20:80 Free-B-Ring flavonoids:flavans), prepared as illustrated in Example 9, were all highly effective at inhibiting the COX activity *in vitro* (Figures 6-8). The inhibition of LOX activity by a flavan extract isolated from *Acacia catechu*, was assessed using a lipoxygenase screening assay *in vitro* as described in Example 4. The results are illustrated in Figure 9. In addition, cell assays that targeted inhibition of compounds in the breakdown of arachidonic acid in the LOX pathway, namely leukotriene B4 were performed using a Soliprin™ sample as described in Example 10. The LTB_4 inhibition results by Soliprin™ are illustrated in Figures 11 and 12.

In vivo efficacy was demonstrated by the application of skin irritating substances, such as AA, to the ears and ankle joint of mice and measuring the reduction of swelling in

mice treated with Soliprin™ as described in Example 11. The results are set forth in Figure 13 and 14. Finally, the efficacy of topical application of Soliprin™ formulation in preventing and treating UV induced skin erythema is illustrated in Example 12 and Figure 15. In the study described in Example 12, Soliprin™ in a blend ratio of 80:20 as of Free-B-Ring flavonoids:flavans was dissolved in water and applied topically at two concentration to the skin of hairless mice both before and after UV exposure, respectively. The erythema scores of the hairless mice from four Soliprin™ groups, in both concentrations and regardless the applications time as before or after UV exposure, all showed much less redness in smaller skin areas as compared to severe and extended erythema in both the control group and the group that was treated with Sooth-A Cain.

Example 13 (Tables 11 and 12) describes a general method for the preparation of a Soliprin™ cream using pharmacologically, dermatologically and cosmetic acceptable excipients. For purposes of illustration this Example provides a detailed procedure for the preparation of both a 0.5 wt % and 1.5 wt % Soliprin™ cream. Finally, both of the Soliprin™ creams prepared as described in Example 13 were evaluated on human skin for potential irritation and induction of contact sensitization. A total of 97 and 101 subjects completed induction and challenge with the 0.5% and 1.5% Soliprin™ creams, respectively. Test results show that Soliprin™ creams at 0.5% and 1.5% concentration produced minimal irritation and did not elicit evidence of induced contact sensitization.

In summary, the present invention includes methods that are effective in simultaneously inhibiting both the COX and LOX enzymes. The method for the simultaneous dual inhibition of the COX and LOX pathways is comprised of administering a composition comprising a mixture of Free-B-Ring flavonoids and flavans synthesized and/or isolated from a single plant or multiple plants to a host in need thereof. The ratio of Free-B-Ring flavonoids to flavans in the composition can be in the range of 99:1 Free-B-Ring flavonoids:flavans to 1:99 of Free-B-Ring flavonoids:flavans. In specific embodiments of the present invention, the ratio of Free-B-Ring flavonoids to flavans is selected from the group consisting of approximately 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. In a preferred embodiment of the invention, the ratio of Free-B-Ring flavonoids:flavans in the composition of matter is approximately 20:80. In a preferred embodiment, the Free-B-Ring flavonoids are isolated from a plant or plants in

the *Scutellaria* genus of plants and flavans are isolated from a plant or plants in the *Acacia* genus of plants.

The present further includes methods for the prevention and treatment of COX and LOX mediated skin diseases and conditions. The method for preventing and treating COX and LOX mediated skin diseases and conditions is comprised of administering to a host in need thereof an effective amount of a composition comprising a mixture of Free-B-Ring flavonoids and flavans synthesized and/or isolated from a single plant or multiple plants together with a pharmaceutically acceptable carrier. The ratio of Free-B-Ring flavonoids to flavans can be in the range of 99:1 Free-B-Ring flavonoids:flavans to 1:99 of Free-B-Ring flavonoids:flavans. In specific embodiments of the present invention, the ratio of Free-B-Ring flavonoids to flavans is selected from the group consisting of approximately 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. In a preferred embodiment of the invention, the ratio of Free-B-Ring flavonoids:flavans in the composition of matter is approximately 20:80. In a preferred embodiment, the Free-B-ring flavonoids are isolated from a plant or plants in the *Scutellaria* genus of plants and flavans are isolated from a plant or plants in the *Acacia* genus of plants.

Applicant believes that U.S. Application Serial No. 10/104,477, filed March 22, 2002, entitled "Isolation of a Dual COX-2 and 5-Lipoxygenase Inhibitor from Acacia," is the first report of a composition of matter isolated from the *Acacia* genus of plants that demonstrates dual specificity for COX and LOX and that U.S. Application Serial No. 10/091,362, filed March 1, 2002, entitled "Identification of Free-B-Ring Flavonoids as Potent COX-2 Inhibitors," is the first report of a correlation between Free-B-Ring flavonoid structure and COX inhibitory activity. These discoveries led to a novel blending of two classes of specific compounds --Free-B-Ring Flavonoids and flavans--to form a composition of matter, referred to herein as Soliprin™, which can be used for the prevention and treatment of COX and LOX mediated diseases and conditions, as described in U.S. Application Serial No. 10/427,746, filed April 30, 2003, entitled "Formulation With Dual Cox-2 And 5-Lipoxygenase Inhibitory Activity." COX and LOX mediated diseases and conditions include, but are not limited to diseases and conditions of the skin including, but are not limited to sun burns, thermal burns, acne, topical wounds, minor inflammatory conditions caused by fungal, microbial and viral infections, vitilago, systemic lupus erythromatosus, psoriasis, carcinoma, melanoma, as well as other mammal

skin cancers, skin damage resulting from exposure to UV radiation, chemicals, heat, wind and dry environments, wrinkles, saggy skin, lines and dark circles around the eyes, dermatitis and other allergy related conditions of the skin. Although not limited by theory, it is believed that the mechanism of action of this class of compounds is the direct dual inhibition of both COX and LOX enzymatic activity.

The present invention further includes therapeutic compositions comprising the therapeutic agents of the present invention including various formulations thereof. Methods for the preparation of these compositions, together with methods for the determination of their purity and specific composition are described in Examples 5-9 and Figure 10.

In a preferred embodiment, the method of prevention and treatment of COX and LOX mediated skin related diseases and conditions according to this invention comprises administering topically to a host in need thereof a therapeutically effective amount of the formulated Free-B-Ring flavonoids and/or flavans isolated from a single source or multiple sources. The purity of the individual and/or a mixture of Free-B-Ring flavonoids and flavans includes, but is not limited to 0.01% to 100%, depending on the methodology used to obtain the compound(s). In a preferred embodiment, doses of the mixture of Free-B-Ring flavonoids and/or flavans containing that same are an efficacious, nontoxic quantity generally selected from the range of 0.001% to 100% based on total weight of the topical formulation. Persons skilled in the art using routine clinical testing are able to determine optimum doses for the particular ailment being treated.

The present invention includes evaluation of the different composition of Free-B-Ring flavonoids and flavan using enzymatic and *in vivo* anti-inflammation models to optimize the formulation and obtain the greatest potency as described below. The present invention provides a commercially viable process for the isolation, purification and combination of *Acacia* flavans with Free-B-Ring flavonoids to yield a composition of matter having desirable physiological activity. In addition to their use for the prevention and treatment of the above described diseases and conditions of the skin, the therapeutic compositions described herein can also be used to sooth sensitive skin and to provide smooth and youthful skin with improved elasticity, reduced and delayed aging, enhanced youthful appearance and texture, and increased flexibility, firmness, smoothness and suppleness.

The compositions of the present invention can be formulated as pharmaceutical compositions which include other components such as a pharmaceutically and/or cosmetically acceptable excipient, an adjuvant, and/or a carrier. For example, compositions of the present invention can be formulated in an excipient that the host to be treated can tolerate. An excipient is an inert substance used as a diluent or vehicle for a drug. Examples of such excipients include, but are not limited to water, buffers, saline, Ringer's solution, dextrose solution, mannitol, Hank's solution, preservatives and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer, tris buffer, histidine, citrate, and glycine, or mixtures thereof, while examples of preservatives include, but are not limited to thimerosal, m- or o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid or solids, which can be taken up in a suitable liquid as a suspension or solution for administration. Thus, in a non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

In one embodiment of the present invention, the composition can also include an adjuvant or a carrier. Adjuvants are typically substances that generally enhance the biological response of a mammal to a specific bioactive agent. Suitable adjuvants include, but are not limited to, Freund's adjuvant; other bacterial cell wall components; aluminum-based salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins; viral coat proteins; other bacterial-derived preparations; gamma interferon; block copolymer adjuvants, such as Hunter's Titermax adjuvant (Vaxcel.TM., Inc. Norcross, Ga.); Ribi adjuvants (available from Ribi ImmunoChem Research, Inc., Hamilton, Mont.); and saponins and their derivatives, such as Quil A (available from Superfos Biosector A/S, Denmark). Carriers are typically compounds that increase the half-life of a therapeutic composition in the treated host. Suitable carriers include, but are not limited to, polymeric controlled release formulations, biodegradable implants, liposomes, bacteria, viruses, oils, esters, and glycols.

In one embodiment, the composition is prepared as a controlled release formulation, which slowly releases the composition of the present invention into the host. As used herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release vehicles will be known to those skilled in the art. Preferred controlled release formulations are biodegradable (i.e., bioerodible).

The therapeutic agents of the instant invention are preferably administered topically by any suitable means, known to those of skill in the art for topically administering therapeutic compositions including, but not limited to as an ointment, gel, lotion, or cream base or as an emulsion, as a patch, dressing or mask, a nonsticking gauze, a bandage, a swab or a cloth wipe. Such topical application can be locally administered to any affected area, using any standard means known for topical administration. A therapeutic composition can be administered in a variety of unit dosage forms depending upon the method of administration. For particular modes of delivery, a therapeutic composition of the present invention can be formulated in an excipient of the present invention. A therapeutic reagent of the present invention can be administered to any host, preferably to mammals, and more preferably to humans. The particular mode of administration will depend on the condition to be treated.

In one embodiment, a suitable ointment is comprised of the desired concentration of the mixture of Free-B-Ring flavonoids and flavans, that is an efficacious, nontoxic quantity generally selected from the range of 0.001% to 100% based on total weight of the topical formulation, from 65 to 100% (preferably 75 to 96%) of white soft paraffin, from 0 to 15% of liquid paraffin, and from 0 to 7% (preferably 3 to 7%) of lanolin or a derivative of synthetic equivalent thereof. In another embodiment the ointment may comprise a polyethylene - liquid paraffin matrix.

In one embodiment, a suitable cream is comprised of an emulsifying system together with the desired concentration of the mixture of Free-B-Ring flavonoids and flavans as provided above. The emulsifying system is preferably comprised of from 2 to 10% of polyoxyethylene alcohols (e.g. the mixture available under the trademark Cetomacrogol™1000), from 10 to 25% of stearyl alcohol, from 20 to 60% of liquid paraffin, and from 10 to 65% of water; together with one or more preservatives, for example from 0.1 to 1% of N,N"-methylenebis[N'-[3-(hydroxymethyl)-2,5-dioxo-4-

imidazolidinyl]urea] (available under the name Imidurea USNF), from 0.1 to 1% of alkyl 4-hydroxybenzoates (for example the mixture available from Nipa Laboratories under the trade mark Nipastat), from 0.01 to 0.1% of sodium butyl 4-hydroxybenzoate (available from Nipa Laboratories under the trade mark Nipabutyl sodium), and from 0.1 to 2% of phenoxyethanol. Example 13 describes the formulation of two different concentrations of the composition of this invention as a cream and Example 14 describes a study undertaken to evaluate the cream for irritation and sensitization of the skin. From this study it was determined that Soliprin™ is a safe composition that can be applied topically at an efficacious concentration without causing irritation or sensitization of the skin.

In one embodiment, a suitable gel is comprised of a semi-solid system in which a liquid phase is constrained within a three dimensional polymeric matrix with a high degree of cross-linking. The liquid phase may be comprised of water, together with the desired amount of the mixture of Free-B-Ring flavonoids and flavans, from 0 to 20% of water-miscible additives, for example glycerol, polyethylene glycol, or propylene glycol, and from 0.1 to 10%, preferably from 0.5 to 2%, of a thickening agent, which may be a natural product, for example tragacanth, pectin, carrageen, agar and alginic acid, or a synthetic or semi-synthetic compound, for example methylcellulose and carboxypolymethylene (carbopol); together with one or more preservatives, for example from 0.1 to 2% of methyl 4-hydroxybenzoate (methyl paraben) or phenoxyethanol-differential. Another suitable base, is comprised of the desired amount of the mixture of Free-B-Ring flavonoids and flavans, together with from 70 to 90% of polyethylene glycol (for example, polyethylene glycol ointment containing 40% of polyethylene glycol 3350 and 60% of polyethylene glycol 400, prepared in accordance with the U.S. National Formulary (USNF)), from 5 to 20% of water, from 0.02 to 0.25% of an anti-oxidant (for example butylated hydroxytoluene), and from 0.005 to 0.1% of a chelating agent (for example ethylenediamine tetraacetic acid (EDTA)).

The term soft paraffin as used above encompasses the cream or ointment bases white soft paraffin and yellow soft paraffin. The term lanolin encompasses native wool fat and purified wool fat. Derivatives of lanolin include in particular lanolins which have been chemically modified in order to alter their physical or chemical properties and synthetic equivalents of lanolin include in particular synthetic or semisynthetic compounds

and mixtures which are known and used in the pharmaceutical and cosmetic arts as alternatives to lanolin and may, for example, be referred to as lanolin substitutes.

One suitable synthetic equivalent of lanolin that may be used is the material available under the trademark Softisan™ known as Softisan 649. Softisan 649, available from Dynamit Nobel Aktiengesellschaft, is a glycerine ester of natural vegetable fatty acids, of isostearic acid and of adipic acid; its properties are discussed by H. Hermsdorf in Fette, Seifen, Anstrichmittel, Issue No. 84, No.3 (1982), pp. 3-6.

The other substances mentioned hereinabove as constituents of suitable ointment or cream bases and their properties are discussed in standard reference works, for example pharmacopoeia. Cetomacrogol 1000 has the formula $CH_3(CH_2)_m(OCH_2CH_2)_nOH$, wherein m may be 15 or 17 and n may be 20 to 24. Butylated hydroxytoluene is 2,6-di-tert-butyl-p-cresol. Nipastat is a mixture of methyl, ethyl, propyl and butyl 4-hydroxybenzoates.

The compositions of the invention may be produced by conventional pharmaceutical techniques. Thus the aforementioned compositions, for example, may conveniently be prepared by mixing together at an elevated temperature, preferably 60-70°C, the soft paraffin, liquid paraffin if present, and lanolin or derivative or synthetic equivalent thereof. The mixture may then be cooled to room temperature, and, after addition of the hydrated crystalline calcium salt of mupirocin, together with the corticosteroid and any other ingredients, stirred to ensure adequate dispersion.

Regardless of the manner of administration, the specific dose is calculated according to the approximate body weight of the host. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art and is within the scope of tasks routinely performed by them without undue experimentation, especially in light of the dosage information and assays disclosed herein. These dosages may be ascertained through use of the established assays for determining dosages utilized in conjunction with appropriate dose-response data.

It should be noted that the invention described herein may be used for veterinary as well as human applications and that the term "host" should not be construed in a limiting manner. In the case of veterinary applications, the dosage ranges can be determined as described above, taking into account the body weight of the animal.

The compositions of this invention can be administered by any method known to one of ordinary skill in the art. The modes of administration include, but are not limited to, enteral (oral) administration, parenteral (intravenous, subcutaneous, and intramuscular) administration and topical application. The method of treatment according to this invention comprises administering internally or topically to a patient in need thereof a therapeutically effective amount of a mixture of Free-B-Ring flavonoids and flavans synthesized and/or isolated from a single plant or multiple plants. In a preferred embodiment the composition is administered topically.

The following examples are provided for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLES

Example 1. Preparation of Organic and Aqueous Extracts from *Acacia* and *Scutellaria* Plants

Plant material from *Acacia catechu* (L) *Willd.* barks, *Scutellaria orthocalyx* roots, *Scutellaria baicalensis* roots or *Scutellaria lateriflora* whole plant was ground to a particle size of no larger than 2 mm. Dried ground plant material (60 g) was then transferred to an Erlenmeyer flask and methanol:dichloromethane (1:1) (600 mL) was added. The mixture was shaken for one hour, filtered and the biomass was extracted again with methanol: dichloromethane (1:1) (600 mL). The organic extracts were combined and evaporated under vacuum to provide the organic extract (see Table 1 below). After organic extraction, the biomass was air dried and extracted once with ultra pure water (600 mL). The aqueous solution was filtered and freeze-dried to provide the aqueous extract (see Table 1 below).

Table 1. Yield of Organic and Aqueous Extracts of *Acacia* and *Scutellaria* Species

Plant Source	Amount	Organic Extract	Aqueous Extract
Acacia catechu barks	60 g	27.2 g	10.8 g
Scutellaria orthocalyx roots	60 g	4.04 g	8.95 g
Scutellaria baicalensis roots	60 g	9.18 g	7.18 g
Scutellaria lateriflora (whole plant)	60 g	6.54 g	4.08 g

Example 2. Inhibition of COX-2 and COX-1 Peroxidase Activity by Plant Extracts from *Acacia catechu*, Various *Scutellaria* Species and Other Plants

The bioassay directed screening process for the identification of specific COX-2 inhibitors was designed to assay the peroxidase activity of the enzyme as described below.

Peroxidase Assay. The assay to detect inhibitors of COX-2 was modified for a high throughput platform (Raz). Briefly, recombinant ovine COX-2 (Cayman) in peroxidase buffer (100 mM TBS, 5 mM EDTA, 1 μM Heme, 1 mg epinephrine, 0.094% phenol) was incubated with extract (1:500 dilution) for 15 minutes. Quantablu (Pierce) substrate was added and allowed to develop for 45 minutes at 25°C. Luminescence was then read using a Wallac Victor 2 plate reader. The results are presented in Table 2.

Table 2 sets forth the inhibition of enzyme by the organic and aqueous extracts obtained from five plant species, including the bark of *Acacia catechu*, roots of two *Scutellaria* species and extracts from three other plant species, which are comprised of structurally similar Free-B-Ring flavonoids. Data is presented as the percent of peroxidase activity relative to the recombinant ovine COX-2 enzyme and substrate alone. The percent inhibition by the organic extract ranged from 30% to 90%.

Table 2. Inhibition of COX-2 Peroxidase Activity by Various Species

Plant Source	Inhibition of COX-2 by organic extract	Inhibition of COX-2 by aqueous extract
Acacia catechu (bark)	75%	30%
Scutellaria orthocalyx (root)	55%	77%
Scutellaria baicalensis (root)	75%	0%
Desmodium sambuense (whole plant)	55%	39%
Eucaluptus globulus (leaf)	30%	10%
Murica nana (leaf)	90%	0%

Comparison of the relative inhibition of the COX-1 and COX-2 isoforms requires the generation of IC_{50} values for each of these enzymes. The IC_{50} is defined as the concentration at which 50% inhibition of enzyme activity in relation to the control is achieved by a particular inhibitor. In these experiments, IC_{50} values were found to range from 6 to 50 μg/mL and 7 to 80 μg/mL for the COX-2 and COX-1 enzymes, respectively, as set forth in Table 3. Comparison of the IC_{50} values of COX-2 and COX-1 demonstrates the specificity of the organic extracts from various plants for each of these enzymes. The organic extract of *Scutellaria lateriflora* for example, shows preferential inhibition of

COX-2 over COX-1 with IC_{50} values of 30 and 80 µg/mL, respectively. While some extracts demonstrate preferential inhibition of COX-2, others do not. Examination of the HTP fractions and purified compounds from these fractions is necessary to determine the true specificity of inhibition for these extracts and compounds.

Table 3. IC_{50} Values of Organic Extracts for Human and Ovine COX-2 and COX-1

Plant Source	IC_{50} Human COX-2 (µg/mL)	IC_{50} Ovine COX-2 (µg/mL)	IC_{50} Ovine COX-1 (µg/mL)
Acacia catechu (bark)	3	6.25	2.5
Scutellaria orthocalyx (root)	Not done	10	10
Scutellaria baicalensis (root)	30	20	20
Scutellaria lateriflora (whole plant)	20	30	80
Eucaluptus globulus (leaf)	Not done	50	50
Murica nana (leaf)	5	6	7

Example 3. Inhibition of COX-1 and COX-2 Peroxidase Activity

In order to screen for compounds that inhibited the COX-1 and COX-2 activities, a high throughput, *in vitro* assay was developed that utilized the inhibition of the peroxidase activity of both enzymes. (Needleman *et al.* (1986) Annu Rev Biochem. 55:69). Briefly, the composition or compound being examined was titrated against a fixed amount of COX-1 and COX-2 enzymes. A cleavable, peroxide chromophore was included in the assay to visualize the peroxidase activity of each enzyme in presence of arachidonic acid as a cofactor. Typically, assays were performed in a 96-well format. Each inhibitor, taken from a 10 mg/mL stock solution in 100% DMSO, was tested in triplicate at room temperature using the following range of concentrations: 0, 0.1, 1, 5, 10, 20, 50, 100, and 500 µg/mL. To each well, 150 µL of 100 mM Tris-HCl, pH 7.5 was added along with 10 µL of 22 µM Hematin diluted in tris buffer, 10 µL of inhibitor diluted in DMSO and 25 units of either the COX-1 or COX-2 enzyme. The components were mixed for 10 seconds on a rotating platform, followed by the addition of 20 µL of 2 mM N,N,N'N'-tetramethyl-p-phenylenediamine dihydrochloride (TMPD) and 20 µL of 1.1 mM arachidonic acid to initiate the reaction. The plate was shaken for 10 seconds and then incubated 5 minutes before reading the absorbance at 570 nm. The inhibitor concentration vs. % inhibition was plotted and the IC_{50} determined by taking the half-maximal point along the isotherm and

intersecting the concentration on the X-axis. The IC_{50} was then normalized to the number of enzyme units in the assay. The results are summarized in Table 4.

Table 4. Inhibition of COX Enzyme Activity by Purified Free-B-Ring Flavonoids

Free-B-Ring Flavonoids	Inhibition of COX-1	Inhibition of COX-2
Baicalein	107%	109%
5,6-Dihydroxy-7-methoxyflavone	75%	59%
7,8-Dihydroxyflavone	74%	63%
Baicalin	95%	97%
Wogonin	16%	12%

The dose responses and IC_{50} values for a standardized Free-B-Ring flavonoid extract, baicalin, and baicalein isolated from the roots of *Scutellaria baicalensis* are provided in Figures 1, 2 and 3, respectively. The dose responses and IC_{50} values for two standardized flavan extract (50% and >90% flavans, respectively) isolated from the heartwood of *Acacia catechu* are provided in Figures 4 and 5, respectively. The dose responses and IC_{50} values for three formulations of Free-B-Ring flavonoids and flavans of varying composition are provided in Figure 6 (80:20 blending), Figure 7 (50:50 blending) and Figure 8 (20:80 blending), respectively.

Example 4. Inhibition of 5-Lipoxygenase by Catechin isolated from *Acacia catechu*

One of the most important pathways involved in the inflammatory response is produced by non-heme, iron-containing lipoxygenases (5-LO, 12-LO, and 15-LO), which catalyze the addition of molecular oxygen onto fatty acids such as AA (AA) to produce the hydroperoxides 5-, 12- and 15-HPETE, which are then converted to leukotrienes. There were early indications that the flavan extract from *A. catechu* may provide some degree of LOX inhibition, thereby preventing the formation of 5-HPETE. A Lipoxygenase Inhibitor Screening Assay Kit (Cayman Chemical, Inc., Cat# 760700) was used to assess whether an extract isolated from *A. catechu* containing >90% flavans directly inhibited LOX *in vitro*. The 15-LO from soybeans normally used in the kit was replaced with potato LOX, after a buffer change from phosphate to a tris -based buffer using microfiltration was performed. This assay detects the formation of hydroperoxides through an oxygen sensing chromagen. Briefly, the assay was performed in triplicate by adding 90 μL of 0.17 units/μL potato 5-LO, 20 μL of 1.1 mM AA, 100 μL of oxygen-sensing chromagen and 10 μL of purified

flavan inhibitor to final concentrations ranging from 0 to 500 μg/mL. The IC_{50} for 5-LO inhibition from this composition was determined to be 1.38 μg/mL/unit of enzyme. The results are set forth in Figure 9.

Example 5. HPLC Quantification of Free-B-Ring Flavonoids in Active Extracts Isolated from *Scutellaria orthocalyx* (roots), *Scutellaria baicalensis* (roots) and *Oroxylum indicum* (seeds)

The presence and quantity of Free-B-Ring flavonoids in five active extracts isolated from three different plant species as described in Examples 1 and 2 were determined by HPLC and the results are set forth in the Table 5, below. The Free-B-Ring flavonoids were quantitatively analyzed by HPLC on a Luna C-18 column (250 x 4.5 mm, 5 μm) using a 1% phosphoric acid and acetonitrile gradient from 80% to 20% in 22 minutes. The Free-B-Ring flavonoids were detected using a UV detector at 254 nm and identified based on retention time by comparison with baicalin, baicalein and other Free-B-Ring flavonoid standards.

Table 5. Free-B-Ring Flavonoid Content in Active Plant Extracts

Active Extracts	**Weight of Extract**	**% Extractible from BioMass**	**Total amount of Free-B-Ring Flavonoids**	**% Free-B-Ring Flavonoids in Extract**
S. orthocalyx (aqueous extract)	8.95 g	14.9%	0.2 mg	0.6%
S. orthocalyx (organic extract)	3.43 g	5.7%	1.95 mg	6.4%
S. baicalensis (aqueous extract)	7.18 g	12.0%	0.03 mg	0.07%
S. baicalensis (organic extract)	9.18 g	15.3%	20.3 mg	35.5%
Oroxylum indicum (organic extract)	6.58 g	11.0%	0.4 mg	2.2%

Example 6. HPLC Quantification of Active Extracts from *Acacia catechu*

The flavans in the organic and aqueous extracts isolated from *Acacia catechu* as illustrated in Examples 1 and 2 were quantified by HPLC using a PhotoDiode Array detector (HPLC/PDA) and a Luna C18 column (250 mm x 4.6 mm). The flavans were eluted from the column using an acetonitrile gradient from 10% to 30% ACN over a period of 20 minutes, followed by 60% ACN for five minutes. The results are set forth in

Table 6. The flavans were quantified based on retention time and PDA data using catechin and epicatechin as standards. The retention times for the two major flavans were 12.73 minutes and 15.76 minutes, respectively.

Table 6. Free-B-Ring Flavonoid Content in Active Plant Extracts

Active Extracts from bark of *A. catechu*	Weight of Extract	% Extractible from BioMass	% Flavans in Extract
Aqueous Extract	10.8 g	18.0%	0.998%
Organic Extract	27.2 g	45.3%	30.37%

Example 7. Preparation of a Standardized Extract from *Acacia catechu*

Acacia catechu (500 mg of ground root) was extracted twice with 25 mL (2 x 25 mL) of the following solvent systems. (1) 100% water, (2) 80:20 water:methanol, (3) 60:40 water:methanol, (4) 40:60 water:methanol, (5) 20:80 water:methanol, (6) 100% methanol, (7) 80:20 methanol:THF, (8) 60:40 methanol:THF. The two extracts from each individual extraction were combined concentrated and dried under low vacuum. The identification of the chemical components in each extract was achieved by HPLC using a PhotoDiode Array detector (HPLC/PDA) and a 250 mm x 4.6 mm C18 column. The chemical components were quantified based on retention time and PDA data using catechin and epicatechin as standards. The results are set forth in Table 7. As shown in Table 7, the flavan extract generated from solvent extraction with 80% methanol/water provided the highest concentration of flavan components.

Table 7. Solvents for Generating Standardized Flavan Extracts from *Acacia catechu*

Extraction Solvent	Weight of Extract	% Extractible from BioMass	Total amount of Catechins	% Catechins in Extract
100% water	292.8 mg	58.56%	13 mg	12.02%
water:methanol (80:20)	282.9 mg	56.58%	13 mg	11.19%
water:methanol (60:40)	287.6 mg	57.52%	15 mg	13.54%
water:methanol (40:60)	264.8 mg	52.96%	19 mg	13.70%
water:methanol (20:80)	222.8 mg	44.56%	15 mg	14.83%
100% methanol	215.0 mg	43.00%	15 mg	12.73%
methanol:THF (80:20)	264.4 mg	52.88%	11 mg	8.81%
methanol:THF	259.9 mg	51.98%	15 mg	9.05%

(60:40)				

Higher purity material can be obtained by recrystallization of extracts having a catechin content of between 8%-15% using an alcohol/water and/or aqueous solvents as the recrystallization solvent. It may be necessary to decolorize prior to recrystallization by adding active charcoal or other decolorization agent to a heated saturated solution of the extract. The high purity catechins then crystallized upon cooling of the heated saturated solution. The crystals were then filtered to remove solvent, dried and ground into a fine powder. Recrystallization can be repeated as necessary to achieve a the desired level of purity (60% - 100% of catechin flavans).

<u>Example 8</u>. <u>Preparation of Standardized Free-B-Ring Flavonoid Extracts from various *Scutellaria* species</u>

Scutellaria orthocalyx (500 mg of ground root) was extracted twice with 25 mL of the following solvent systems. (1) 100% water, (2) 80:20 water:methanol, (3) 60:40 water:methanol, (4) 40:60 water:methanol, (5) 20:80 water:methanol, (6) 100% methanol, (7) 80:20 methanol:THF, (8) 60:40 methanol:THF. The extracts were combined, concentrated and dried under low vacuum. Identification of chemical components in each extract was performed by HPLC using a PhotoDiode Array detector (HPLC/PDA) and a 250 mm x 4.6 mm C18 column. The chemical components were quantified based on retention time and PDA data using baicalein, baicalin, scutellarein, and wogonin as standards. The results are set forth in Table 8.

Table 8. Quantification of Free-B-Ring Flavonoids Extracted from *Scutellaria orthocalyx*

Extraction Solvent	Weight of Extract	% Extractible from BioMass	Total amount of Flavonoids	% Flavonoids in Extract
100% water	96 mg	19.2%	0.02 mg	0.20%
Water:methanol (80:20)	138.3 mg	27.7%	0.38 mg	0.38%
Water:methanol (60:40)	169.5 mg	33.9%	0.78 mg	8.39%
Water:methanol (40:60)	142.2 mg	28.4%	1.14 mg	11.26%
Water:methanol (20:80)	104.5 mg	20.9%	0.94 mg	7.99%

100% methanol	57.5 mg	11.5%	0.99 mg	10.42%
methanol:THF (80:20)	59.6 mg	11.9%	0.89 mg	8.76%
methanol:THF (60:40)	58.8 mg	11.8%	1.10 mg	10.71%

Scutellaria baicalensis (1000 mg of ground root) was extracted twice using 50 mL of a mixture of methanol and water as follows: (1) 100% water, (2) 70:30 water:methanol, (3) 50:50 water:methanol, (4) 30:70 water:methanol, (5) 100% methanol. The extracts were combined, concentrated and dried under low vacuum. Identification of the chemical components was performed by HPLC using a PhotoDiode Array detector (HPLC/PDA), and a 250 mm x 4.6 mm C18 column. The chemical components in each extract were quantified based on retention time and PDA data using baicalein, baicalin, scutellarein, and wogonin standards. The results are set forth in Table 9.

Table 9. Quantification of Free-B-Ring Flavonoids Extracted from *Scutellaria baicalensis*

Extraction Solvent	Weight of Extract	% Extractible from BioMass	Total amount of Flavonoids	% Flavonoids in Extract
100% water	277.5 mg	27.8%	1 mg	0.09%
Water:methanol (70:30)	338.6 mg	33.9%	1.19 mg	11.48%
Water:methanol (50:50)	304.3 mg	30.4%	1.99 mg	18.93%
Water:methanol (30:70)	293.9 mg	29.4%	2.29 mg	19.61%
100% methanol	204.2 mg	20.4%	2.73 mg	24.51%

Higher purity Free-B-Ring flavonoids can be obtained by recrystallization of extracts having a Free-B-Ring flavonoid content of between 8-15% using alcohol/water as a recrystallization solvent. It may be necessary to decolorize prior to recrystallization by adding active charcoal or other decolorization agent to a heated saturated solution of the extract. The Free-B-Ring flavonoids crystallized upon cooling. The crystals were filtered, dried and ground into a fine powder. Recrystallization can be repeated as necessary to achieve a the desired level of purity (60% - 100% of Free-B-Ring flavonoids).

Example 9. Preparation of a Formulation with a Standardized Free-B-Ring Flavonoid Extract from the Roots of *Scutellaria baicalensis* and a Standardized Flavan Extract from the Bark of *Acacia catechu*

A novel composition of matter, referred to herein as Soliprin™ was formulated using two standardized extracts isolated from *Acacia* and *Scutellaria*, respectively, together with one or more excipients. A general example for preparing such a composition is set forth below. The *Acacia* extract used in this example contained >80% total flavans, as catechin and epicatechin, and the *Scutellaria* extract contained >80% Free-B-Ring flavonoids, which was primarily baicalin. The Scutellaria extract also contained other minor amounts of Free-B-Ring flavonoids as set forth in Table 11. One or more excipients/preservatives was also added to the composition of matter. The ratio of flavans and Free-B-Ring flavonoids can be adjusted based on the indications and the specific requirements with respect to inhibition of COX vs. LO, requirements of skin penetration, and potency requirements of the product, such as duration of potency required, etc. The quantity of the excipients can be adjusted based on the actual active content of each ingredient. A blending table for each individual batch of product must be generated based on the product specification and QC results for individual batch of ingredients. Additional amounts of active ingredients in the range of 2-5% are recommended to meet the product specification.

Scutellaria baicalensis root extract (38.5 kg) (lot # RM052302-01) having a Free-B-Ring flavonoid content of 82.2% (baicalin); *Acacia catechu* bark extract (6.9 kg) (lot # RM052902-01) with total flavan content of 80.4%; and excipient (5.0 kg of Candex) were combined to provide a Soliprin™ formulation (50.4 kg) having a blending ratio of 85:15 by weight of the active Free-B-Ring flavonoids and flavans. Table 10 provides the quantification of the active Free-B-Ring flavonoids and flavans of this specific batch of Soliprin™ (Lot#G1702-COX-2), determined using the methods provided in Examples 6 and 8. With reference to Table 10, this specific batch of Soliprin™ contains 86% total active ingredients, including 75.7% Free-B-Ring flavonoids and 10.3% flavans. Figure 10 illustrates the HPLC chromatogram of a representative Soliprin™ sample which had a blending ratio of 80:20 by weight of the active Free-B-Ring flavonoids and flavans.

Table 10. Free-B-Ring Flavonoid and Flavan Content of a Soliprin^{TM} Formulation

Active Components	% Content
1. **Flavonoids**	
a. Baicalin	62.5%
b. Minor Flavonoids	
i. Wogonin-7-glucuronide	6.7%
ii. Oroxylin A 7-glucuronide	2.0%
iii. Baicalein	1.5%
iv. Wogonin	1.1%
v. Chrysin-7-glucuronide	0.8%
vi. 5-Methyl-wogonin-7-glucuronide	0.5%
vii. Scutellarin	0.3%
viii. Norwogonin	0.3%
ix. Chrysin	<0.2%
x. Oroxylin A	<0.2%
c. **Total Free-B-ring Flavonoids**	**75.7%**
2. **Flavans**	
a. Catechin	9.9%
b. Epicatechin	0.4%
c. **Subtotal Flavans**	**10.3%**
3. **Total Active Ingredients**	**86%**

Using the same approach, the following batches of Soliprin^{TM} were prepared using a combination of a standardized Free-B-Ring flavonoid extract from *Scutellaria baicalensis* roots and a standardized flavan extract from *Acacia catechu* bark having a blending ratio of 12:88 and 15:85, respectively.

Scutellaria baicalensis root extract (58.0g) (lot # RM021203-01) having a Free-B-Ring flavonoid content of 87.9% (as baicalin) and *Acacia catechu* bark extract (442.0g) (lot # RM050603-01) with total flavan content of 84.9% were blended to provide a Soliprin^{TM} composition (500 g, lot#QJ205-19) having a blending ratio of 12:88 by weight. Utilizing the methods provided in Examples 6 and 8, the Free-B-Ring flavonoid content of (baicalin) was 9.65% and flavan content (total catechin and epicatechin) was 73.2% in this specific batch of SoliprinTM (lot#QJ205-19).

Scutellaria baicalensis root extract (300g) (lot # RM060403-01) having a Free-B-Ring flavonoid content of 82.9% (as baicalin) and *Acacia catechu* bark extract (1700g) (lot # RM050603-01) with total flavan content of 90.8% were blended to provide a Soliprin^{TM} composition (2000 g, lot#A1904) having a blending ratio of 15:85 by weight. Utilizing the

methods provided in Examples 6 and 8, the Free-B-Ring flavonoid content (baicalin) was 15.6% and flavan content (total catechin and epicatechin) was 75.0% in this specific batch of Soliprin™ (lot#A1904).

Example 10. Measurements of Dose Response and IC_{50} Values of 5-LO Enzyme Inhibition from a Formulation of Soliprin™

A Soliprin™ formulation (80:20) was prepared as described in Example 9. (See also Example 14 of U.S. Pat. Application Serial No. 10/427,746, filed April 30, 2003, entitled "Formulation With Dual COX-2 And 5-Lipoxygenase Inhibitory Activity," which is incorporated herein by reference in its entirety) using a combination of a standardized Free-B-Ring flavonoid extract from *Scutellaria baicalensis* roots and a standardized flavan extract from *Acacia catechu* bark with a blending ratio of 80:20. The sample was titrated in tissue culture media containing THP-1 or HT-29 cells; monocyte cell lines that express COX-1, COX-2 and 5-LOX. A competitive ELISA for Leukotriene B4 (LTB4; Neogen, Inc., Cat#406110) was used to assess the effect of this Soliprin™ formulation on newly synthesized levels of LTB4 present in each cell line as a measure Soliprin™ 's inhibitory effect on the 5-LOX pathway. The assay was performed in duplicate by adding 160,000 to 180,000 cells per well in 6-well plates. The Soliprin™ formulation was added to the THP-1 cultures at 3, 10, 30 and 100 μg/mL and incubated overnight (~12-15 hrs) at 37°C with 5% CO_2 in a humidified environment. The results are set forth in Figure 11, which shows that the production of newly LPS-induced LTB4 was almost completely inhibited by the addition of Soliprin™ to theTHP-1 cultures between 3 and 10 μg/mL.

Soliprin™ and ibuprofen, another known 5-LOX inhibitor, were added to the HT-29 cells at 3 μg/mL and incubated 48 hrs at 37°C with 5% CO_2 in a humidified environment. Each treated cell line was then harvested by centrifugation and disrupted by gentle dounce homogenization lysis in physiological buffers. As shown in Figure 12, Soliprin™ inhibited generation of 80% of the newly synthesized LTB4 in HT-29 cells. Ibuprofen only showed a 20% reduction in the amount of LTB4 over the same time period.

Example 11. Evaluation of the Efficacy of Soliprin™ with *in vivo* Mouse Ear Swelling Model

A Soliprin™ formulation was prepared using a combination of a standardized Free-B-Ring flavonoid extract from *Scutellaria baicalensis* roots and a standardized flavan extract from *Acacia catechu* bark with a blending ratio of 80:20 as described in Example 9. To test whether this composition could be used to treat inflammation *in vivo*, the composition was administered by oral gavage to 4-5 week old ICR mice (Harlan Labs) one day before treatment of their ears with arachidonic acid (AA). Test mice were fed dose equivalents of 50, 100 and 200 mg/kg of Soliprin™ suspended in olive oil while control mice were fed only olive oil. The following day, 20 μL of 330 mM AA in 95% alcohol was applied to one ear of each mouse, while alcohol was applied to the other ear as a control. Mice treated with Soliprin™ showed a measurable dose response that tracked with increasing doses of Soliprin™, as demonstrated in Figure 13. With reference to Figure 13, the 200 mg/kg dose reduces swelling by over 50% as compared to the "No treatment" control. The 50 mg/kg dose of Soliprin™ was as effective as the 50 mg/kg dose of another strong anti-inflammatory, indomethacin.

In another animal model designed to demonstrate the anti-inflammatory activity of Soliprin™ the 80:20 formulation described above was orally administrated to mice in a dose of 100 mg/kg suspended in olive oil ~12 hours before injection of 20 μL of 100 mM AA in 95% ethanol into the hind ankle joints of 4-5 week old ICR mice (Harlan Labs). The test group was fed the Soliprin™ formulation, while another group was not given the formulation. Control groups included mice that had not received arachidonic acid injections (negative control) and a group that had 95% ethanol without AA injected (vehicle control). These groups were also not given Soliprin™. The results are set forth in Figure 14. With reference to Figure 14, the mice given Soliprin™ that were injected with AA showed background levels of swelling as compared to the controls and the untreated arachidonic injected group. These results demonstrate the effectiveness of Soliprin™ for reducing swelling in joints, the site of action.

Example 12. Evaluation of the Efficacy of Soliprin™ in Preventing and Treating Damage Resulting from Exposure of Skin to UV radiation

Six groups of hairless female mice (five mice per group) (Strain SKH-1, Harlan Labs) were irradiated, while anesthetized, for three minutes on three consecutive days with 0.626 mW/cm^2 to test the effectiveness of the Soliprin™ formulation in preventing and treating damage resulting from exposure of skin to UV radiation. The Soliprin™ formulation was prepared using a combination of a standardized Free-B-Ring flavonoid extract from *Scutellaria baicalensis* roots and a standardized flavan extract from *Acacia catechu* bark with a blending ratio of 80:20 as described in Example 9. The six treatment groups were as follows:

Group #	
1	**Control group**: no treatment *before* or *after* UV irradiation
2	**Positive control**: treated with a topical application of Sooth-A-Caine (Banana Boat) *after* UV irradiation
3	**Soliprin™ Treatment B-1**: treated with topical application of 1 mg/mL Soliprin™ in water *before* UV irradiation
4	**Soliprin™ Treatment A-1**: treated with topical application of 1 mg/mL Soliprin™ in water *after* UV irradiation
5	**Soliprin™ Treatment B-2:** treated with topical application of 5 mg/mL Soliprin™ in water *before* UV irradiation
6	**Soliprin™ Treatment A-2:** treated with topical application of 5 mg/mL Soliprin™ in water *after* UV irradiation

After three days of UV exposure and treatment, the mice were scored on level of erythema (redness) using the following scale: 0- no visible erythema; 1- very slight erythema; 2- well defined erythema; 3- severe erythema; and 4- tumor formation. Erythema was scored by eye for each group. The results are set forth in Figure 15. With reference to Figure 15 it can be seen that the control group (Group 1) had severe redness on day 3 (72 hours after the three day exposure to UV radiation). The Sooth-a-caine group also had maximum redness on day 3 (Group 2). The redness for the Soliprin™ treated groups (Groups 3-6) never exceeded a score of 2. These scores, though subjective, show that Soliprin™ is effective in both preventing and treating UV caused skin erythema.

Photographs of representative mice on day four clearly demonstrate differences between the control group, the Sooth-a-cain™ treated groups and the Soliprin™ treated groups (data not shown). The control group and Sooth-a-cain™ treated animals exhibited very extensive patterns and redness of erythema compared to the animals treated with the Soliprin™ formulation both before and after UV exposure. The animals treated before UV irradiation with 5 mg/mL Soliprin™ exhibited the least amount of erythema as compared to all of the other animals.

Example 13. Formulation of the Soliprin™ Composition into a Cream

Two different concentrations of Soliprin™ (0.5% and 1.5% by weight of Soliprin™) (lot#A1904 as described in Example 9) were formulated as creams as illustrated in the following procedures and in Tables 11 and 12.

Soliprin™ (Lot#A1904) was dissolved in water at room temperature and homogenized with a blender until it was fully dispersed in solution (approximately 5 minutes). At room temperature and without stirring or agitating the solution, Ultrez-21 carbomer was added by sprinkling onto the surface of the solution and allowing it to fully wet (no white areas visible) and fall into the solution. With gentle stirring, the solution was then heated to 40^{0}C and glycerin was added (Part A). The mixture was then stirred for an additional 5 minutes. The remaining components (Part B) were weighed and heated to 40^{0}C while mixing. At 40^{0}C, the remaining components (Part B) were added to Part A and the resulting composition was mixed well until homogenous (approximately 5 minutes). The emulsion was cooled to 30^{0}C and the pH was adjusted to approximately 5.5 (5.3 to 5.7) by titrating with neutralizer while stirring with a stir bar and/or spatula. The emulsion became highly viscous due to neutralization-induced conformational change of the carbomer. The emulsion eventually achieved a suitable viscosity for an emulsion cream. The emulsion cream was then mixed until uniform after which it was poured into a clean storage vessel and stored at 2^0 to 8^{0}C for one month.

Table 11. Ingredient list for a 0.5% Soliprin Cream

Phase	Ingredient	% (w/w)	Weight (g)
Aqueous	Water, Purified	85.00	1275.0
	Soliprin (Lot#A1904)	0.50	7.5
	Ultrez 21 Carbomer	0.50	7.5

	Glycerin	8.00	120.0
Oil	PEG-7 Glyceryl Cocoate	3.00	45.0
	Caprylic/ Capric Triglyceride	2.67	40.0
PH Neutralizer	Sodium Hydroxide (18% w/v), Molecular Biology Grade	0.00	0.0
SUM	7 Ingredients	99.7	1495.0

Table 12. Ingredient list in a 1.5% Soliprin Cream

Phase	Ingredient	% (w/w)	Weight (g)
Aqueous	Water, Purified	84.00	1260.0
	Soliprin (Lot#A1904)	1.50	22.5
	Ultrez 21 Carbomer	0.50	7.5
	Glycerin	8.00	120.0
Oil	PEG-7 Glyceryl Cocoate	3.00	45.0
	Caprylic/ Capric Triglyceride	2.67	40.0
pH Neutralizer	Sodium Hydroxide (18% w/v), Molecular Biology Grade		
SUM	7 Ingredients	99.7	1495.0

Example 14. Evaluation of a Soliprin™ Cream for Irritation and Induction of Contact Sensitization by Repetitive Application to Human Skin.

The Soliprin™ was tested on human skin using an adaptation of the Draize Patch Test (Marzulli and Maibach (1977) Contact Allergy: Predictive Testing in Humans. In Advances in Modern Toxicology, Dermatotoxicology and Pharmacology. Eds. Marzulli, F.N and Maibach, H.I. 4, 353-372). The test sites were located on the upper arm or the paraspinal region of the back. Each test article had an induction site and a challenge site. The induction site was comprised of two sub-sites: an original-site and a move-site. Patches, which contains 0.2ml of Soliprin cream on each patch, were applied repeatedly to the original-site unless a sufficiently strong irritation reaction developed, requiring the patch to be applied to the move-site. Patches were applied by a clinical research institute and were removed and discarded by the subjects approximately 24 or 48/72 hours later. In the induction phase, repetitive application of the test article to the same site on the skin and a total of 9 induction patches were applied within a 4-week period. The rest period was 10 to 21 days between application of the last induction patch and application of the challenge patch. During this time no test article or any other material was applied to the

test area. At the challenge phase, the test article was applied to a naive site on the opposite side of the body and discarded by the subjects approximately 24 or 48 hours later.

Skin responses to each patch application were examined and graded under light supplied by a 100-watt incandescent blue bulb according to the designated scoring scale. In instances where a strong irritation reaction warranted application of the test article to the move-site, residual scores were be recorded through the end of induction (or until resolved if reactions persist after induction is completed) for all previously exposed sites. All skin reactions were recorded. During the challenge phase, skin responses were evaluated approximately 48 and 72 or 96 hours after patch application. Conclusions, with regard to induced sensitivity, were derived primarily from the challenge evaluations.

The two Soliprin™ creams prepared in the Example 13 at 0.5% and 1.5% Soliprin™ concentrations were evaluated according to the above protocol. A total of 120 subjects were recruited for each group. Ninety-seven subjects completed the study for the 0.5% Soliprin™ group and 101 subjects completed the study for 1.5% Soliprin™ group. There was no evidence of sensitization reaction for either the 0.5% and 1.5% Soliprin™ creams. For the 0.5% Soliprin™, during induction, sixteen subjects exhibited occasional occurrences of slight to mild erythema (scores of + and/or 1). At challenge, four subjects exhibited slight to mild erythema at 48 hours that cleared by 96 hours. For 1.5% Soliprin™, during induction, twenty-six subjects exhibited occasional occurrences of slight to mild erythema (scores of + and/or 1). At challenge, one subjects exhibited slight to mild erythema at 48 hours that cleared by 96 hours.

This study demonstrates that Soliprin™ is a safe ingredient that can be applied topically to human skin at an efficacious concentration without causing irritation or sensitization.